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NUTRITIONAL EVALUATION OF METHANOL-BASED YEAST SINGLE-CELL PROTEIN FOR GROWING PULLETS, LAYING HENS AND REPRODUCING CHICKENS

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Nutritional evaluation of methanol-based yeast single-cell protein for growing pullets, laying hens and reproducing chickens

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INTRODUCTION

Yeast has been used in baking bread, brewing beer, and making wine for some time. It has also been used as a water soluble vitamin source for animals as well as for humans. But it was not until 1942 that the potential of yeast as a protein source for animals was recognized by animal scientists (Braude, 1942). Since then, the world has developed increasing interest in yeast because conventional methods of food production, such as farming and animal husbandry, have had increasing difficulty in keeping pace with the ever-growing demand for protein.

The protein obtained from yeast has been named single-cell protein (SCP). SCP refers to either a crude or a refined source of protein whose origin is unicellular or simple multicellular in structure. Examples of microorganisms used for SCP production include yeast, algae, bacteria, and protozoa. These organisms have the ability to grow at a very rapid exponential rate under optimum substrate supply and favorable fermentation chamber conditions. Besides growing rapidly, these organisms have the ability to utilize inorganic nitrogen for amino acid synthesis and to use a variety of substrates (agriculture wastes, gas-oil, n-paraffin, n-alkane, methanol, ethanol, acetate and CO_2) as an energy source. These abilities of the microorganisms make SCP production free from environmental uncertainties, while not competing with conventional agriculture for raw materials or land.

Among the organisms, yeast has received the most attention for SCP production because yeast products are accepted generally as food sources

for humans, and the organism has been grown successfully on several substrates on a pilot plant scale (Laskin, 1977). In addition, yeast single-cell protein (YSCP) usually contains relatively high levels of protein (56 to 67%) and is a rich source of B vitamins.

The use of by-products (n-paraffin, n-alkanes, gas-oil or methanol) as substrates for the production of YSCP creates some concern about the carryover of undesirable substances in the final product to be used in feeding programs. Therefore, the nutritional and toxicological characteristics of YSCP grown on waste materials need to be evaluated before YSCP's use in animal or human diets can be recommended. During the last decade, the nutritional and toxicological properties of several YSCPs have been described in reviews by Snyder (1970), Waslien (1975), VanderWal (1976), Vananuvat (1977), Tannanbaum and Cooney (1978), and Garattini et al. (1979).

The initial portion of this dissertation will review the literature cited above as well as recent publications concerning nutritional and toxicological characteristics of several YSCPs as a protein source for chickens. The remaining portion will deal with specific experiments designed to determine the nutritional qualities of a YSCP grown on methanol.

LITERATURE REVIEW

The chemical composition of several YSCPs will be examined in this Literature Review. This will include proximate analyses as well as mineral, vitamin and crude fiber composition assays, and fatty acid profiles. Additionally, the chemical composition of YSCP will be compared with the composition of conventional protein sources. Secondly, nutritive value of YSCP for chickens as a source of amino acids, as an energy source and as a phosphorus source will be examined. Finally, the palatibility problem associated with YSCP, and the toxicological aspect of YSCP will be scrutinized.

Chemical Composition of YSCP

Results of proximate analyses show that YSCP sources contain a higher proportion of crude protein (nitrogen x 6.25) than do plants (Table 1). YSCP nitrogen content ranges from 9.1 to 10.6%. This variation in the concentration of nitrogen has been postulated to be related to many factors such as the type of microorganisms, the nutrient mediums, the general growth conditions and the technology of biomass preparation after the fermentation process (Schulz and Oslage, 1976).

Because of YSCP's single-cell structure which results in a high proportion of nucleus to cell mass, YSCP is high in nucleic acids (Table 2). Although nitrogen from nucleic acids is included in total crude protein content of YSCP, this nonprotein nitrogen is of little nutritional

Table 1. Chemical composition (per cent of dry matter)

	YSCP[a]				BSCP[b]		
	Torpina[c] L gas-oil	Torpina[c] G n-paraffin	Liquipron[d] n-alkane	SIR LI70[e] methanol	Pruteen[c] Methanol	Soybean[c] Meal	Fishmeal[c]
Dry matter	94.5	95.5	93.7	-	96.9	87.5	91.0
Ash	7.5	6.0	12.3	7.2	11.5	5.7	15.5
Organic matter	87.0	89.5	-	-	85.4	81.8	75.5
Nitrogen	10.0	9.6	9.1	10.0	12.5	7.2	10.2
Crude protein	66.2	60.0	57.1	62.5	78.1	45.0	66.2
Amino acid N	53.2	47.0	-	-	57.6	38.0	54.2
Nucleic acid	13.0	13.0	-	-	20.5	7.0	12.0
Crude fiber	-	-	3,4	1.1	-	6.0	-
Crude fat	1.0	9.0	2.5	-	4.9	1.0	8.1
NFE[f]	19.8	20.0	24.6	24.2	2.4	29.8	1.2

[a] Yeast single-cell protein.

[b] Bacterial single-cell protein.

[c] VanderWal (1976).

[d] Russo et al. (1976).

[e] Cardini et al. (1976).

[f] Nitrogen free extract.

Table 2. Nucleic acid content of SCP[a]

SCP/substrate	RNA	DNA	Total[b]	Protein[c]
Yeast/n-paraffin	8.2	0.4	8.6	13
Yeast/n-paraffin	6.7	0.5	7.2	15
Yeast/whey	9.3	0.5	9.8	15
Yeast/methanol	7.7	0.8	8.5	21
Yeast/n-alkane	5.4	1.0	6.4	16
Yeast/sulfite waste liquor	11.2	0.7	11.9	22
Bacteria/methanol	16.5	1.7	18.2	22

[a]Schulz and Oslage (1976).

[b]As per cent of dry matter.

[c]As percent of total protein.

significance under normal feeding situations (D'Mello, 1979). However, nucleic acids are a rich source of phosphorus and supply substantial amounts of an available form of phosphorus to diets (Burns and Baker, 1976).

YSCP contains a higher ash than does SBM (Table 1). The high ash content of YSCP indicates that YSCP may be a good source of several minerals. The detailed analysis of the minerals presented in Table 3 shows that these YSCPs contain relatively high levels of potassium, magnesium, phosphorus, zinc and sulfur. In contrast, YSCP contains low levels of sodium, calcium and selenium. Considerable variation has been observed in the levels of specific minerals in various YSCP's seemingly because of differences in the amounts of minerals added to the growth medium (Mannino and Cavazzoni, 1980). YSCPs, however, consistently contain a high level of phosphorus because this mineral is a component of the nucleic acids inherent in yeast.

Besides being a rich source of crude protein and minerals, YSCP is recognized for its richness in most water soluble vitamins (Table 4). The crude fat content of YSCPs varies considerably but frequently exceeds that of SBM (Table 1). The fatty acid composition of YSCP fat differs greatly from that of SBM fat even though the ratios of unsaturated to saturated fatty acids in these two materials are similar (Table 5). The fat of YSCP contains relatively high levels of fatty acids with 15 or 17 carbon atoms; whereas, the fat of SBM contains fatty acids with 14, 16 and 18 carbon atoms. The nutritional and toxicological significance of the odd-numbered carbon chain fatty acids of YSCP will be discussed later in this Literature Review.

Table 3. Mineral composition of YSCP (ppm)

Element	Organism and Substrate							
	S. cerevisiae[a]	C. lipolytica[a]		C. boidini[a]				
	Baker yeast	glucose	n-paraffin	glucose	xylose	ethanol	methanol	Soybean meal[b]
Potassium	20,100	10,800	11,700	20,500	19,200	10,400	15,500	20,020
Sodium	592	116	78	74	91	64	63	2,500
Magnesium	1,246	572	1,088	2,760	1,739	1,395	2,636	-
Calcium	392	294	135	253	245	373	434	2,700
Phosphorus	23,800	29,300	30,500	39,500	34,200	35,200	39,600	6,200
Iron	56	66	112	149	120	109	253	-
Copper	5	8	4	12	15	31	21	15
Zinc	294	59	59	193	167	135	204	16
Manganese	5	3	3	4	5	4	7	43
Cobalt	1.4	1.3	1.5	2.2	1.5	1.8	2.1	-
Chromium	0.7	1.7	3	2.9	3.4	1.1	5.1	-
Cadmium	0.3	0.5	0.8	1.3	2.5	1.2	2.6	-
Lead	3	6	6.6	3.4	4.9	5	5.3	-
Silicon	8	19	26	20	29	33	33	-
Sulfur	2,100	1,200	1,800	2,100	2,000	1,600	1,700	-
Selenium[c]	-	-	-	-	-	-	-	0.1

[a]Mannino and Cavazzoni (1980).

[b]National Research Council (1977).

[c]Selenium content of these YSCPs was not reported. However, a YSCP grown on n-paraffin contained 14 ppb Se (Jackson and Kirkpatrick, 1978).

Table 4. Water soluble vitamin B content of some YSCP as compared with fish meal and alfalfa meal (mg/100 g dry matter)

	Torpina[a] L	Torpina[a] G	Torula[a]	Brewer[a] yeast	Fish meal[b]	Alfalfa meal[c]
B_1	0.3	0.4	0.7	17.0	0.05	0.34
B_2	18.3	18.3	4.6	4.7	0.49	1.36
Niacin	12.5	43.0	41.0	45.0	5.50	3.80
B_6	5.7	2.5	3.8	4.7	0.40	0.65
Folic acid	0.7	0.6	6.8	2.1	0.06	0.42
Pantothenic acid	1.0	12.5	5.0	8.0	0.09	0.25
Biotin	0.01	0.01	0.22	0.06	0.02	0.03
Inositol	143.0	326.0	-	-	-	-
B_{12}	0.01	0.01	-	-	0.01	-

[a]Vananuvat (1977).

[b]International Feed No. 1-00-023 (NRC, 1977).

[c]International Feed No. 5-02-009 (NRC, 1977).

Table 5. Fatty acid profile of YSCP (per cent of total fatty acids)

Fatty acid	YSCP and substrate			SBM[a]	Fishmeal[a]
	SIR-LI70[a] methanol	Liquipron[b] n-alkane	Torpina G[c] n-paraffin		
14:0[d]	0.28	1.50	1.96	-	10.20
14:1	-	0.20	-	-	-
15:0	0.39	3.60	7.21	-	0.91
15:1	-	0.61	-	-	-
16:0	21.88	10.60	10.49	23.40	41.58
16:1	18.72	10.60	8.21	-	10.48
17:0	1.70	6.30	2.85	-	1.43
17:1	2.03	27.90	26.16	-	0.55
17:3	-	3.10	-	-	-
18:0	1.38	1.30	1.72	4.10	6.93
18:1	23.06	19.80	11.90	12.20	20.93
18:2	29.89	11.90	13.30	57.20	1.22
18:3	-	2.60	2.82	3.0	-
Even number[e]	95.54	58.50	58.18	100.0	96.92
Odd number	4.46	41.50	41.82	-	3.08
Unsaturated	73.70	76.70	75.77	72.50	37.68
Saturated	26.30	23.30	24.23	27.50	62.32

[a] Cardini et al. (1976).

[b] Bizzi et al. (1976).

[c] Bernardini et al. (1976).

[d] Number of carbon atoms and number of double bonds.

[e] Per cent of total.

Table 6. Amino acid content of YSCP as compared with SBM and fish meal (g/16g of N)

	YSCP[a]		
	Torpina L gas-oil	Lavera[d] gas-oil	Torpina G[e] n-paraffin
Lysine	7.8	8.4	8.2
Methionine (cystine)	2.5	1.4(.87)	1.1 (1.5)
Arginine	5.0	5.3	5.5
Histidine	2.1	2.0	2.2
Isoleucine	5.3	5.5	5.1
Phenylalanine and tyrosine	8.8	8.3	7.5
Threonine	5.4	5.1	5.0
Tryptophan	1.3	-	-
Valine	5.8	5.6	5.8
Glycine and Serine	-	9.5	10.5

[a]Yeast single-cell protein.

[b]Bacterial single-cell protein.

[c]VanderWal (1976).

[d]El Boushy and Roodbeen (1980).

[e]Jackson and Kirkpatrick (1978).

[f]Russo et al. (1976).

[g]Succi et al. (1980).

[h]White and Balloun (1977).

[i]International Feed No. 5-04-612 (NRC, 1977).

[j]International Feed No. 5-02-009 (NRC, 1977).

			BSCP[b]		
Liquipron[f] n-alkane	SIR LI70[g] methanol	Yeast[h] methanol	Pruteen[c] methanol	SBM[i]	Fishmeal[j]
8.4	7.7	6.5	5.5	6.5	8.0
1.6	1.5(2.0)	1.1(.62)	3.1	1.5(1.5)	2.9(.9)
6.3	5.3	3.3	4.7	7.6	6.3
2.5	2.5	2.5	1.9	2.7	2.4
5.3	5.6	5.0	3.9	5.3	4.7
8.3	7.3	6.8	6.3	7.9	7.4
4.5	8.1	8.0	6.2	8.5	7.4
5.6	4.8	3.5	4.2	3.9	4.1
1.6	1.7	0.8	0.8	1.4	1.1
6.4	5.9	5.6	4.8	5.6	5.3
-	10.2	7.5	-	10.7	10.6

YSCP's crude fiber content also differs from that of vegetable products. The crude fiber portion of YSCP does not consist of hemicellulose, cellulose and lignin as do the fibers of vegetable products. Rather, the major fraction of the yeast cell wall consists of glucan (a poly-sacchrides of 1,3 bonded glucose residue), a mannan-protein complex, and a small amount of chitin. The carbohydrates of the yeast cell consist of mannon and glucan, with some α1, 1 trehalose (Sibereisen, 1960; cited in Schulz and Oslage, 1976). Yeast also contains mannose and glucose that have "true digestibility coefficients" of 71 and 81% respectively (Shannon and McNab, 1973).

Nutritive Value of YSCP for Chickens

Primarily, YSCP will be used as a source of amino acids for chickens; however, metabolizable energy and phosphorus contents of YSCP indicate that the material also would supply energy and an available form of phosphorus in chickens' diets.

YSCP as a source of amino acids

The amino acid concentrations reported for several YSCPs, bacterial single-cell protein (BSCP), SBM and fishmeal are presented in Table 6. The amino acids listed have been limited to those categorized as dietary essentials for growing chicks. In general, the amino acid profile of YSCP is similar to that of SBM. Both contain relatively high levels of lysine and low levels of sulfur-containing amino acids. These two protein sources differ, however, in arginine content with YSCP containing a much

lower level of this amino acid than SBM.

Research conducted with broiler chicks fed YSCP as the only source of protein in semipurified diets has shown that methionine was the first limiting amino acid and that arginine was the second limiting amino acid in YSCP (Daghir and Sell, 1980). Likewise, deficiencies of methionine and arginine have also been found in practical rations containing YSCP as a source of protein for broiler chicks (VanderWal, 1976; White and Balloun, 1977). Sell et al. (1981) also reported that, on a calculated basis, arginine deficiency is more pronounced in the starter diet than in the grower diet when YSCP was substituted for 75% of the protein of the SBM contained in these diets.

Waldroup and Hazen (1975), Shannon et al. (1976), Jackson and Kirkpatrick (1978) and Yoshida (1979) evaluated several YSCPs as an amino acid source for replacement pullets, laying hens, and reproducing chickens and showed that, on the basis of performance of chickens, neither methionine nor arginine was deficient in the YSCP diets.

YSCP as an energy source

In addition to providing amino acids, YSCP will also provide a substantial amount of metabolizable energy (ME) to the chicken's diet. Most YSCPs contain a higher ME content than that found in SBM (2440 kcal ME/kg) (Table 7). The high ME content of YSCP seems to be associated with the high lipid content of YSCP (Table 5) which has a true digestibility coefficient of 73% (Shannon & McNab, 1973). The ME content of YSCP for chickens ranges from 2200 to 3380 kcal/kg (Table 7). The wide range in

Table 7. Metabolizable energy content of YSCP for poultry

	Substrate	ME kcal/kg	Reference
Candida sp.	n-paraffin	3380	Yoshida (1979)
Candida sp.	n-paraffin	2850	Yoshida (1979)
Candida sp.	n-paraffin	3110	Yoshida (1979)
Pichia sp.	n-paraffin	2510	Yoshida (1979)
Candida sp.	n-paraffin	3050	VanderWal (1976)
Candida sp.[a]	n-paraffin	3045 (2716)	Shannon and McNab (1973)
	gas-oil	2550	VanderWal (1976)
Candida sp.	methanol	2945	Succi et al. (1980)
Unknown	methanol	2200	Bales (1980)
Candida sp.	acetic acid	3840	Yokata et al. (1976)
Candida sp.	molasses	3080	Yokata et al. (1976)

[a]Most ME values are classical except for one presented in parentheses, which is nitrogen corrected.

the ME content of YSCP is probably related to the substrate and species of yeast used in the production of the various YSCPs.

YSCP as a phosphorus source

Another potential advantage of using YSCP in the diets of chickens is that it contains a relatively high level of phosphorus as compared with protein sources of vegetable origin. Burns and Baker (1976), Yoshida (1979), and Ashraf and Sell (1981) have shown that, in comparison with reference sources of phosphorus, the relative availability of phosphorus from YSCP ranges from 59 to 103%. These relative availabilities are considerably higher than that of SBM (33%) (Scott et al., 1976).

In general, the Literature Review suggests that nutritive values of YSCP are comparable to those of SBM, provided that diets are adequately supplemented with methionine, arginine and Se. The lack of consistent nutritive values of several YSCPs, namely ME content (2200 to 3380 kcal/kg), available phosphorus (59 to 103%) and crude protein content (56 to 67%), may be related to the substrate on which the YSCPs were grown, the specie and genus of yeast tested, or the production technique used to separate the YSCP from the culture media. Therefore, the nutritive value of one YSCP should not be used to estimate the nutritive value of another.

Palatability Aspect of YSCP for Chickens

Waldroup et al. (1971) evaluated a YSCP grown on n-alkane as a source of amino acids for broiler chicks. In these trials, YSCP constituted up to

30% of the diets in all mash- or pelleted form. The chicks fed all-mash diets containing 15% or more YSCP had a significant reduction in feed intake. On the other hand, feed intake of chicks fed up to 30% YSCP in a pelleted diet was not affected. Depression in feed intake has also been observed in several other studies in which YSCP was fed to broiler chicks in all-mash diets (VanderWal, 1976; White and Balloun, 1977; Bales, 1980; and Sell et al. (1981).

The adverse effect of YSCP on feed intake by chickens may be related to the physical texture of the diets. YSCP is characteristically dry in texture and powdery in consistency. Consequently, all-mash diets containing substantial levels of YSCP acquire these same characteristics. Seemingly, because of the dry, powdery texture of YSCP diets, feed consumption of chicks is impaired unless corrective measures are taken. Waldroup et al. (1971), Vanderwal (1976) and White and Balloun (1977) reported that feeding YSCP-containing diets as pellets overcame the adverse effects of dietary YSCP on feed intake by chickens. These results support the contention that the palatability problem associated with YSCP is one of physical texture of the diets and is not associated with a "chemical characteristic" of this protein source.

Toxicological Aspects of YSCP

Although the nutritional characteristics of YSCP grown on an unconventional substrate seem favorable as compared with those of conventional sources, the nutritional quality factor alone will not be sufficient to obtain permission from regulatory agencies such as the

Food and Drug Administration (FDA), for use of YSCP in animal production. Producers of YSCP grown on ethanol or wood hydrosalate have been granted permission by the FDA to produce these YSCPs and market them as a protein source to be used in the diets of humans. But the FDA is reluctant to sanction the use of YSCP grown on hydrocarbons (n-paraffin, n-alkane or gas-oil) (Tannenbaum and Cooney, 1978) or methanol, even when the substrates are actually of food grade. The FDA requires data obtained with a specific YSCP material and a particular animal showing that, in a toxicological sense, routine use of the YSCP in diets will have no adverse effects. Several experiments designed to scrutinize the toxicological properties of YSCP grown on methanol or hydrocarbon have been conducted according to the protocol designed by the FDA and the Protein Advisory Group of the United Nations (De Groot, 1976; Ashraf et al., 1981; and Ashraf and Vetter, 1981).

Ashraf et al. (1981) evaluated YSCP grown on methanol as a protein source for rats over three generations and showed that substitution of YSCP for 100% of the dietary SBM protein (23% of the diet) had no adverse effects on the reproductive performance of rats. On the other hand, when YSCP from the same source was fed in a semipurified diet at dietary levels of 69% to female rats over a reproductive cycle, there was a decline in weaning weight of pups and in maintanance of body weight during lactation by dams. But no adverse effects on newborn pups in terms of physical abnormality or number of live pups born were observed with the high level of YSCP in the diet (69%) (Ashraf and Vetter, 1981). However,

in both of these studies, female rats fed YSCP diets had a higher incidence of mineral deposits at the corticomedullary junction of the kidneys (nephrocalcinosis) than those fed the control diet. The nephrocalcinosis was attributed to a calcium-to-phosphorus ratio of less than one in YSCP diets. Diets with a Ca:P ratio of less than one have been reported to induce nephrocalcinosis in female rats (De Groot, 1976).

Results of experiments conducted by Ashraf et al. (1981) and Ashraf and Vetter (1981) agree with the results of a series of long-term experiments conducted by De Groot (1976). De Groot (1976) reported results of a two year study in which rats were fed diets containing 0, 10, 20, or 30% YSCP grown on gas-oil or n-paraffin. The study showed no adverse effect on mortality, growth rate, hematology, urine composition or kidney functions. However, serum glutamic pyruvic transaminase and alkaline phosphatase activities of rats fed YSCP were relatively high but were within a normal range. No lesions were observed in the liver of rats fed YSCP nor were there any dietary differences in the type and incidence of tumors. Similarly, the multiple generation phase of the study revealed no effects on fertility, the number of young or the growth and mortality of the young rats during lactation. The study also showed no effects of dietary treatments on carcinogenicity, teratogenicity or mutagenicity in rats and mice.

The long-term studies with rats suggested that the YSCP based on gas-oil, n-paraffin or methanol may be used in the diets of rats without any

adverse effects. In like manner, long-term toxicological experiments have been conducted with chickens. Yoshida (1979) evaluated a YSCP grown on n-paraffin in which the YSCP was fed to chickens for five generations at the dietary level of 15%. The results showed no adverse effects of YSCP on the reproductive performance. Satisfactory results have also been observed in terms of laying hen performance when hens were fed YSCP grown on n-paraffin at dietary levels of up to 12% over eleven, 28-day periods (Jackson and Kirkpatrick, 1978). However, VanderWal (1976) reported that fertility was reduced slightly (3%) when chickens were fed diets containing 10 or 14% YSCP, but hatchability of eggs was not affected.

Although the chemical constituents of YSCP do not differ greatly from those of cereal protein sources, the lipid fraction of hydrocarbon-grown YSCP deserves consideration. The lipid fraction of the YSCPs contains large amounts of odd-chain fatty acids (OCFA) (Table 5). Similarly, OCFA have been found in several foods of animal origin such as fish, bovine milk and fat, and also in human milk (Jeanrenaud, 1965; Garton, 1967; Masoro, 1968; and Mannering, 1971).

Kishimoto et al. (1973) found branched-chain fatty acids and OCFA in the nervous system of a patient with deranged vitamin B_{12} metabolism. These were, likewise, found in patients suffering from Refsum's disease (Lough, 1975; and Hansen, 1965). These findings have led to the concern that feeding an animal a diet containing YSCP high in OCFA may increase OCFA in animal tissues and, consequently, may produce adverse effects. Bizzi et al. (1976) reported results of an experiment in which rats,

chickens and monkeys were fed diets containing a YSCP high in OCFA. These animals showed an accumulation of OCFA in adipose tissue, hearts, liver and platelets, but not to the same extent in the brain. It was indicated also that animals fed a control diet had a measurable amount of OCFA in their tissue, even when the diet had no detect levels of OCFA.

In spite of an accumulation of OCFA, no abnormality was observed in the function of any organs studied. It was concluded that the presence of OCFA in the body at concentrations not exceeding 10% of total fatty acids was compatible with normal physiological function (Bizzi et al., 1976).

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SECTION I. AVAILABILITY OF PHOSPHORUS FROM YEAST SINGLE-CELL PROTEIN FOR GROWING CHICKS

AVAILABILITY OF PHOSPHORUS FROM YEAST SINGLE-CELL PROTEIN FOR GROWING CHICKS

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ABSTRACT

An experiment was conducted to determine the relative availability of phosphorus from methanol-based, yeast single-cell protein (YSCP). Chicks, starting at eight days of age, were fed either tricalcium phosphate (TCP) or YSCP as a source of supplemental phosphorus. The experiment was 14 days in length. The basal diet consisted primarily of corn and isolated soy protein and contained .29% total phosphorus and .76% calcium. The TCP was substituted for limestone, whereas YSCP was substituted for isolated soy protein and cellulose. Each phosphorus source was used in the diet to provide .08, .16, .24 and .32% supplemental phosphorus. Weight gain, percentage femur ash and femur ash were used as criteria of response to dietary phosphorus, and regression analysis was used to evaluate each of these parameters as a function of phosphorus intake. Among the parameters measured, weight of femur ash was most sensitive to change in dietary phosphorus intake. The linear regression equations for TCP and YSCP were $Y = 94.83 + .106X$ and $101.52 + .074X$, respectively, where Y = mg of femur ash and X = mg P intake from TCP or YSCP. Availability of phosphorus from YSCP was estimated according to the slope-ratio technique. The relative availability of phosphorus from YSCP was 69.8% as compared with 100% for TCP.

INTRODUCTION

Concern about a shortage of protein sources for animals has resulted in the development of several alternate sources of proteins such as single-cell protein (SCP). SCP refers to a crude or refined protein whose origin is unicellular or simple multicellular in structure. Organisms used for this purpose include algae, bacteria, protozoa and yeast. Yeasts have been produced successfully on a large scale on several hydrocarbon substrates (Laskin, 1977). Yeast single-cell protein (YSCP) also has been well accepted as a protein source in rations of swine (Russo et al., 1976) and poultry (Vananuvat, 1977).

YSCP is characteristically high in nucleic acid (Schulz and Oslage, 1976) which, in turn, is intrinsically high in phosphorus (Burns and Baker, 1976). The research reported here was conducted to determine for growing chicks the availability of phosphorus from a YSCP grown on methanol.

MATERIALS AND METHODS

Male broiler chicks were used for the bioassay of phosphorus availability from YSCP. The YSCP tested was produced on a methanol substrate and was provided by a commercial company.

The chicks were housed in electrically heated battery brooders with raised wire floors and were given continuous light throughout the experiment. All chicks were fed a positive control diet containing .61% total phosphorus and .76% calcium from one to eight days of age. On day eight, the chicks were divided into 27 experimental units, with each unit consisting of a pen of eight chicks. There were nine dietary treatments and three pens were assigned randomly to each dietary treatment.

A basal diet (Table 1) was formulated to contain .29% total phosphorus and .76% calcium. Graded levels of the supplemental source of phosphorus were added to the basal diet to obtain various concentrations of dietary phosphorus. Tricalcium phosphate (TCP) was used as a standard phosphorus source because the relative biological value of phosphorus in this material is considered to be 100% (Scott et al., 1976). TCP was substituted for limestone at levels of .4, .81, 1.2 and 1.6% of the diet. YSCP was substituted for appropriate quantities of isolated soy protein and cellulose to obtain levels of 3.5, 7.1, 10.7 and 14.3% of the diet. On a calculated basis, each test material contributed .08, .15, .24 and .32% phosphorus to the diet. Laboratory analysis (A. O. A. C., 1975) showed, however, the increments of TCP increased phosphorus levels by .11, .17, .30 and .43%. Similarly, phosphorus levels of YSCP diets increased by .07, .24, .31 and

.41% with incremental changes in YSCP. All the diets were formulated to contain approximately 3050 kcal of ME/kg, 21.5% crude protein and .76% calcium. The crude protein, calcium and phosphorus concentrations in the YSCP were 62, .1 and 2.8%, respectively.

During the 14-day experimental period, weight gain and feed consumption were recorded weekly. At the end of the experiment, three chicks were selected randomly from each pen. The chicks were killed by cervical dislocation, and the left femur was removed. The femurs were heated at 70°C for eight hours to facilitate removal of adherent muscle tissue. Fat was extracted from the femur with a mixture of ethyl ether and methanol (70:30) for four hours in a Goldfisch apparatus. The fat-free bones were dried at 70°C for 12 hours and ashed at 600°C for eight hours.

Weight gain, percentage bone ash (percentage of fat- and moisture-free bone), and total weight of femur ash were used as criteria for phosphorus availability. Regression analysis was done to assess the relationship between mg of phosphorus intake from each phosphorus source and body weight gain, percentage bone ash, or mg femur ash (Snedecor & Cochran, 1967).

The availability of phosphorus from YSCP was estimated by the slope-ratio technique. This involved a comparison of the regression coefficients relating the response of chicks to increments of phosphorus supplied either as YSCP or as TCP.

Table 1. Composition of basal diet used in the assay

	%
Corn	61.78
Isolated soy protein	17.60
Wood cellulose[a]	11.80
Dehydrated alfalfa meal (17% protein)	3.60
Fat[b]	2.40
Tricalcium phosphate[c]	.00
YSCP[d]	.00
Limestone	1.70
Mineral premix[e]	.22
Vitamin premix[f]	.45
DL methionine	.45
	100.00

[a]Solka-Flok, Brown and Company, Berlin, NH.

[b]Blended animal-vegetable fat from a commercial source.

[c]Tricalcium phosphate, Stauffer Chemical Co., New York, N.Y.

[d]The YSCP contained 62, .1 and 2.8% (air-dry basis) crude protein, calcium and phosphorus, respectively.

[e]The premix provided the following per kg of diet: Mn, 51 mg; Zn, 29 mg; Fe, 27 mg; Cu, 4 mg; iodized salt, 1914 mg.

[f]The premix provided the following per kg of diet: vitamin A, 6750 IU; vitamin D_3, 1350 IU; vitamin E, 5.4 IU; vitamin B_{12}, 18 µg; vitamin K, .9 mg; riboflavin, 5.4 mg; calcium pantothenate, 19.8 mg; niacin, 67.5 mg; choline chloride, 360 mg.

RESULTS AND DISCUSSION

Weight gain, percentage bone ash and total weight of femur ash data are presented in Table 2. Quantitative phosphorus intakes from TCP or YSCP were calculated from feed intake data. The regression equations were calculated relating weight gain, percentage bone ash and quantities of femur ash to supplemental phosphorus intake.

When data across all phosphorus intakes from TCP and YSCP were used, the linear and quadratic components of the equations were significant ($P<.05$) for all the criteria of TCP and the percent femur ash for YSCP. On the other hand, only the linear component of regression equations relating weight gain and total femur ash weight to quantitative phosphorus intakes from YSCP were significant. Further analysis showed that the quadratic components of the TCP and YSCP regression equations became negligible if data for the highest level of supplemental TCP and YSCP were eliminated. Consequently, a second set of regression equations was calculated using data obtained with diets containing .11, .17 and .30 or .07, .24 and .31% supplemental phosphorus from TCP or YSCP, respectively (Table 2). Because the latter equations show that the effects of both sources of supplemental phosphorus on all criteria conformed to the linear model required for best use of the slope-ratio technique, this information was used to determine relative availability of phosphorus from YSCP.

Selection of the most appropriate criterion to determine phosphorus availability was made on the basis of relative response to changes in phosphorus intakes. A comparison of the relative responses (percent

Table 2. Performance of chicks fed TCP or YSCP as sources of phosphorus

Diet	Calculated Supplemental Phosphorus,%	Supplemental[a] Levels of Phosphorus,%	Supplemental Phosphorus Consumption (mg)	Feed[b] Intake/ Chick	Gain/[b] Chick	Percent[c] Femur Ash	Femur[c] Ash(mg)
1	.0	-	-	428±10[d]	112±7	22.21±.14	96±6
2	.08	.11	516	470±22	153±4	27.66±.49	142±5
3	.16	.17	892	525±19	173±4	33.39±.28	199±2
4	.24	.30	1542	514±9	174±3	36.34±.10	256±6
5	.32	.43	2313	538±29	173±4	37.50±.40	255±9
6	.08	.07	331	473±12	144±2	27.35±.42	135±7
7	.17	.24	1226	511±26	167±8	30.19±.30	186±6
8	.25	.31	1587	512±12	185±13	34.23±.10	222±15
9	.34	.41	2263	552±15	207±6	36.13±.10	267±14

[a]Supplemental phosphorus levels were determined by laboratory analysis. These values were used to calculate the quantitative consumption of supplemental phosphorus.

[b]Average for triplicate groups of eight male chicks for the experimental period of 8-21 days post hatching. Average initial weight was 70 g per chick.

[c]Average for triplicate groups of three male chicks each. Analyses were done on fat- and moisture-free bone.

[d]Means±SE.

Table 3. Regression equations for the criteria measured as a function of supplemental phosphorus intake[a]

Criteria	Source	Regression Equation	Change[b] %	R^2 Value
Weight gain (g)	TCP	124.27+.039X	.031	.73
	YSCP	120.25+.041X	.034	.79
Percent femur ash[c]	TCP	22.96+.009X	.039	.83
	YSCP	23.29+.006X	.025	.87
Femur ash (mg)	TCP	94.83+.106X	.112	.97
	YSCP	101.52+.074X	.073	.91

[a]Data representing the highest supplemental phosphorus level (.4%) were excluded from the regression analyses.

[b]Percent change per mg phosphorus intake.

[c]Percent of fat- and moisture-free bone.

change per mg phosphorus intake) showed that increments of phosphorus intakes elicited the greatest change in amounts of femur ash, irrespective of phosphorus source (Table 3). Therefore, femur ash was selected as the criterion for determination of relative availability of phosphorus from YSCP.

Relative availability was calculated by dividing the regression coefficient from the YSCP regression equation with that from the TCP equation.

$$\text{Relative availability} = \frac{.074}{.106} \times 100 = 69.8\%$$

Even though the magnitudes of responses were relatively low in the case of percent femur ash, the relative availability of phosphorus obtained using regression coefficients of this parameter (67%) agreed closely with the one obtained from quantitative femur ash data. Previously, Burns and Baker (1976) reported that the phosphorus of another yeast product was 70% as available for chicks as that from potassium phosphate.

The results reported here indicate that, although YSCP may be used primarily as a source of dietary protein, this material also would supply a moderately available form of phosphorus to diets.

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SECTION II. NUTRITIONAL EVALUATION OF YEAST SINGLE-CELL PROTEIN AS A PROTEIN SOURCE FOR LAYING HENS

NUTRITIONAL EVALUATION OF YEAST SINGLE-CELL PROTEIN AS A PROTEIN SOURCE FOR LAYING HENS

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ABSTRACT

Two experiments were conducted to evaluate yeast single-cell protein (YSCP) grown on methanol as a protein source for laying hens. In both experiments, YSCP was substituted for 0, 33, 66 or 100% of the protein of soybean meal (SBM). The experiments were conducted over eleven 28-day periods. Pooled data for seven periods in experiment 1 showed that inclusion of YSCP in the diets resulted in a decrease in average egg weight, egg yield, feed consumption and body weight gain. Rate of egg production and feed efficiency were not affected by the dietary treatments. At the end of the seventh period, methionine supplementation was increased to .13, .22 and .28% in diets containing 6.55, 12.90 and 19.15% YSCP, respectively. This resulted in an improvement in egg yield for the hens fed 6.55 or 12.9% YSCP protein diets, but the hens fed 19.15% YSCP protein diet showed no improvement in egg yield. Body weight gain was improved by increasing the methionine levels of the YSCP diets. In experiment 1, mortality increased with increments of dietary YSCP. This relationship was postulated to be the result of a deficiency of selenium (Se). Experiment 2 was designed to determine the effects of Se supplementation of YSCP-containing diets on laying hen performance. The dietary treatments consisted of a 2 x 4 factorial arrangement of two levels of Se (0 and .1 ppm) and four levels of dietary YSCP (0, 6.5, 12.7 and 18.7%). These dietary levels of YSCP replaced 0, 33, 66 and 100% of the protein of SBM. Total sulfur amino acid levels of the diets containing 0, 6.55, 12.7 and 18.7% YSCP were .62, .67, .71 and .75%, respectively. Neither

level of YSCP nor supplemental Se had a significant effect on laying hen performance except for feed intake. Hens fed diets supplemented with Se consumed significantly more feed than those fed non supplemented diets. No relationship was observed between the levels of YSCP or supplemented Se and rate of mortality. The results indicate that YSCP can be used successfully in diets of laying hens when the diets are supplemented with adequate amounts of methionine.

INTRODUCTION

In recent years much effort and research has been directed toward the development of alternative sources of protein for animals. This has led to the production of single-cell protein (SCP). Organisms used as SCP sources include algae, bacteria, protozoa and yeast. The ability of these organisms to transform simple hydrocarbons such as n-paraffins, n-alkane, ethanol and methanol into microbial protein has stimulated several industries to develop technology for the mass production of SCP. The use of methanol as a substrate for SCP offers considerable technological advantages: namely, ease of handling and economical availability. Because of its volatile characteristics, methanol separates easily from SCP as compared with other possible substrates such as n-paraffin or n-alkanes (Cooney and Makiguchi, 1977; and Dimmling, 1976). Yeast has been produced successfully on a pilot plant scale using substrates such as n-paraffins, n-alkane, ethanol and methanol (Laskin, 1977).

The results of several experiments have shown that YSCP grown on n-paraffin or n-alkane can be used to replace 50 to 100% of a conventional source of protein in laying hen diets (Shannon et al., 1976; Jackson and Kirkpatrick, 1978; and Waldroup and Hazen, 1975).

Previous research done with a YSCP produced in a methanol-based medium indicated that this protein source could be used successfully in diets for growing chicks when supplemental methionine was used or when the diets were pelleted (White and Balloun, 1977). Tegbe and Zimmerman (1977), Slagle and Zimmerman (1979), and Ashraf et al. (1981) found that

YSCP from the same manufacturer was satisfactory for growth of pigs and for growth and reproduction of rats when amino acid and selenium deficiencies were corrected.

After the above research was conducted with the YSCP, the manufacturer changed the YSCP production process slightly. The experiments reported herein were performed to determine the value of YSCP produced by the modified process as a source of dietary amino acids for laying hens.

MATERIALS AND METHODS

General Procedure

Twenty-six-week-old, Single Comb White Leghorn (SCWL) hens of a commercial strain were used. The hens were housed in standard wire cages, 25.4 cm wide and 40.6 cm deep, with two birds per cage. A group of four cages (eight hens) was designated as an experimental unit. Feed and water were supplied ad libitum, and fourteen hours of light per day were provided to the hens throughout the experiments.

Both experiments were 308 days in duration and consisted of 11 periods of 28 days each. Egg production and mortality were recorded daily. Post-mortem examination of dead birds was done by a veterinarian at the Iowa State University Veterinary College to determine the cause of death. Body weights were recorded at the time of housing and at the end of the third, sixth, ninth and eleventh 28-day periods. Feed consumption was recorded for each 28-day period and eggs produced during the last three days of each period were weighed.

Experiment 1

Chemical composition of YSCP used is presented in Table 1. Four diets were tested to evaluate YSCP as a source of protein for SCWL hens. The hens were fed all-mash diets containing SBM, YSCP or combinations of SBM and YSCP. Two hundred and twenty-four hens were allotted randomly to 28 experimental units. Each unit consisted of eight birds. Seven experimental units were assigned randomly to each dietary treatment.

Table 1. Composition of YSCP used in formulating diets in experiments 1 and 2[a]

	%
Dry matter	95.5
Protein crude	61.0
Protein, true	55.0
Nucleic acid	6.0
Ash	11.5
Ether extract	0.8
Ether extract (Acid hydrolysis)	5.0
Fiber	0.2
Methionine	0.64
Cystine	0.35
Lysine	4.08
Arginine	2.76
Glycine + Serine	4.88
Histidine	1.43
Isoleucine	2.28
Leucine	2.44
Phenylalanine + tyrosine	4.37
Threonine	2.65
Valine	2.63
Tryptophan	0.46
Calcium	0.10
Phosphorus	2.80
Se, ppm	0.03

[a]Analyses were provided by the producer of YSCP.

The control diet was composed primarily of corn and SBM (Table 2). Three additional test diets were obtained by substituting YSCP for 33, 66 or 100% of the protein of SBM. The levels of YSCP in the diets were 0, 5.66, 12.90 and 19.15%, respectively. The diets were formulated to be isocaloric (2972 ME kcal/kg) and isonitrogenous (16.67% crude protein). The ME value of YSCP used in the diet formulation was 2900 kcal/kg. The composition of YSCP was 61.0% crude protein, 55% true protein, .1% calcium and 2.8% phosphorus. The true protein value of YSCP was used to formulate the diet. YSCP also was used as a source of phosphorus in the diets (Table 2). This was done because Ashraf and Sell (1981) showed that phosphorus from the YSCP was relatively available (70%) in comparison with phosphorus from tricalcium phosphate.

At the end of the experiment, two hens were selected randomly from each experimental unit and were killed. The liver, heart, proventriculus, pancreas, right cecum, lungs and kidneys were excised from each hen and weighed.

Egg yield (g/hen/daily) was calculated by multiplying average egg weight times the number of eggs produced and dividing this product by the number of hen days.

All the production criteria were subjected to statistical analyses using a split-plot-in-time-and-space as described by Steel and Torrie (1960) wherein the main plot was dietary treatments and the subplot was periods. Data for organ weights and body weights were subjected to analyses of variance. The data on percentage mortality were transformed

Table 2. Composition of diets used in experiment 1

Ingredients	Level of YSCP (%)[a]			
	00	6.55	12.90	19.15
Corn	64.75	65.71	67.16	67.88
SBM	22.50	15.00	7.20	0.00
YSCP	0.00	6.55	12.90	19.15
Wood cellulose[b]	0.00	0.75	1.55	2.35
Fat	2.50	2.00	1.45	1.10
Limestone	7.00	7.51	8.02	8.35
Di-calcium phosphate	2.38	1.57	0.76	0.00
Vitamin premix[c]	0.50	0.50	0.50	0.50
Mineral premix[d]	0.30	0.30	0.30	0.30
DL Methionine	0.07	0.11	0.16	0.19
	100.00	100.00	100.00	100.00
Protein, %	16.67	16.66	16.65	16.69
ME/kg	2972	2972	2971	2972
Methionine, %	0.36	0.39	0.43	0.45
Cystine, %	0.26	0.23	0.20	0.17
TSAA, %[e]	0.62	0.62+.02[f]	0.63+.06	0.62+.09
T. Phos., %	0.76	0.73	0.70	0.67
Calcium, %	3.30	3.29	3.28	3.27
Se, mg/kg	0.052	0.051	0.045	0.041

[a]Inclusion of YSCP at the dietary levels of 0, 6.55, 12.90 and 19.15% replaced 0, 33, 66 and 100% of the protein of SBM, respectively.

[b]Solka-Floc, Brown and Company, Berlin, NH.

[c]Supplied the following per kg of diet: vitamin A, 8000 IU; vitamin D_3, 2400 IU; vitamin B_{12}, 5 µg; riboflavin, 6.6 mg; pantothenate, 6.6 mg; niacin, 22 mg; elhoxyquin, 11 mg.

[d]Supplied the following per kg of diet: Mn, 20 mg; iodized salt, 2.997 g.

[e]Total sulfur amino acids.

[f]Additional supplemental methionine was added during 8 through 11 periods.

to arcsin and subjected to analysis of variance. The statistical analyses were done according to procedures described by Steel and Torrie (1960).

Experiment 2

The results of experiment 1 showed a positive relationship between increasing levels of dietary YSCP and mortality. This relationship was postulated to be related to a deficiency of Se in YSCP. Therefore, experiment 2 was designed to determine the effects of Se supplementation of YSCP-containing diets on laying hen performance.

The experiment was of a completely randomized design with a 2 x 4 factorial arrangement of two levels of dietary Se (0 and .1 ppm) and four levels of dietary YSCP (0, 6.5, 12.7 and 18.70%). One hundred and ninety-two SCWL hens were assigned randomly to 24 experimental units, and three units were allotted randomly to each dietary treatment.

The diets (Table 3) were formulated as described in experiment 1 with two exceptions: 1) the ME value of the YSCP used for ration formulation was reduced from 2900 kcal ME/kg to 2200 kcal ME/kg. This was done because Bales (1980) determined experimentally that the ME value of the YSCP for chickens was 2200 kcal ME/kg. 2) The diets containing YSCP were supplemented with additional DL methionine. Consequently, the total sulfur amino acid (TSAA) levels of diets containing 0, 6.5, 12.7 and 18.6% YSCP were .62, .67, .71, or .75%, respectively.

Statistical analyses of the data were done as described in experiment 1.

Table 3. Composition of basal diets used in experiment 2

Ingredient	Levels of YSCP (%)[a]			
	00	6.50	12.70	18.60
Corn	64.55	66.10	68.10	69.50
SBM	22.50	14.80	7.10	-
YSCP	0.00	6.40	12.70	18.60
Fat	2.50	2.50	2.15	2.16
Limestone	7.20	7.76	8.23	8.60
Di-calcium phosphate	2.38	1.58	0.67	0.00
Vitamin premix[b]	0.50	0.50	0.50	0.50
Mineral premix[c]	0.30	0.30	0.30	0.30
DL Methionine	0.07	0.16	0.25	0.34
	100.00	100.00	100.00	100.00
Protein, %	16.65	16.67	16.67	16.65
ME/kg	29.65	29.66	29.62	2968
Methionine, %	0.36	0.44	0.51	0.58
Cystine, %	0.26	0.23	0.20	0.17
TSAA, %	0.62	0.67	0.71	0.75
T.Phos	0.76	0.74	0.71	0.71
Calcium, %	3.31	3.33	3.31	3.30
Se, mg/kg	0.052	0.048	0.043	0.041

[a]Inclusion of YSCP at the dietary levels of 0, 6.50, 12.7, and 18.6 replaced 0, 33, 66 and 100% of the protein of SMB, respectively.

[b]Supplied following per kg of diet: Vitamin A, 8000 IU; Vitamin D_3, 2400 IU; Vitamin B_{12}, 5 µg; riboflavin, 6.6 mg; pantothenate, 6.6 mg; niacin, 22 mg; ethoxyquin, 11 mg.

[c]Supplied following per kg of diet: Mn, 20 mg; iodized salt, 2.997 g.

RESULTS

Experiment 1

Production data were summarized and analyzed statistically at the end of each 28-day period of experiment 1. A pattern of diet treatment effect on laying hen performance was observed during the first seven periods. As the level of YSCP increased in the diet, rate of egg production decreased numerically, and egg weight and egg yield decreased significantly ($P<.01$) (Table 4). Feed consumed per bird also declined as dietary YSCP increased, but feed efficiency (kg feed/kg eggs) was not affected. Hens fed diets containing 6.5, 12.7 or 18.6% YSCP consumed 4, 8 or 8% less feed and produced 3, 4 or 8% less egg mass, respectively, than those fed diets containing no YSCP (Table 4).

It was postulated that the adverse effect of dietary YSCP on feed intake was the underlying cause of reduced performance of the hens observed through seven periods. Examination of the quantitative intakes of essential nutrients showed that the TSAA consumption of hens fed YSCP may have been inadequate. On a calculated basis, the average daily quantities of TSAA consumed were 609, 585, 572 and 560 mg per hen for hens fed 0, 6.55, 12.9 and 19.5% YSCP, respectively. According to the National Research Council (1977) and Reid and Weber (1974), the lowest TSAA intake (560 mg/hen/daily) should have been sufficient to support satisfactory performance. On the other hand, Scott et al. (1976) recommended an average TSAA intake of 610 mg/hen daily.

Table 4. Effect of YSCP on the performance of SCWL hens, experiment 1[a]

	Levels of dietary YSCP %			
	00	6.55	12.90	19.15
Hen-day egg production, %	80.0±1.4[b]	78.7±1.6	78.7±1.3	77.6±1.2
Egg weight, g**	59.6± .5	59.0± .5	57.8± .5	57.6± .6
Egg yield, g/hen/day**	47.8±1.1	46.4±1.1	45.8± .9	44.0± .9
Feed consumed, g/hen/day**	98.2±1.2	94.4±1.2	90.8±1.2	90.5±1.2
Feed efficiency, kg feed/kg eggs	2.1± .04	2.1± .04	2.0± .03	2.1± .03

[a]Means of seven experimental units per treatment for 7, 28-day periods.

[b]Means ± SE.

**Significant linear effect of levels of YSCP ($P<.01$).

In view of the uncertainties about the TSAA requirement of laying hens and the lack of consistent information on the availability of TSAA from YSCP, supplemental methionine levels were increased to .13, .22 and .28% of diets containing 6.55, 12.9, and 19.5% YSCP, respectively, for periods 8 through 11. These levels of supplemental methionine were selected on the basis of overcoming the dietary methionine and cystine deficits on a molar basis. During the preceding periods, supplementation was done on a weight basis.

The patterns of treatment effects on rate of egg production, egg weight, and feed consumption were not changed notably by additional methionine supplementation during periods 8 through 11 (Table 5). Egg yield and feed efficiency from hens fed diets containing 6.55 and 12.9% YSCP, however, seemed to be improved by extra methionine. Conversely, hens fed the 19.15% YSCP diet produced less egg yeild than hens fed other diets. This inconsistent pattern of response during the 8 to 11 period interval resulted in a significant quadratic effect of diets on egg yield (Table 5).

Gain in body weight during periods 1 through 7 was lower for hens fed diets containing 12.9 and 19.5% YSCP than for hens fed 0 or 6.55% YSCP (Table 6). These hens gained an average of 80 g less than those on other dietary treatments. There was no significant effect of YSCP level on weight gain during periods 8 through 11, suggesting that the extra supplemental methionine used during the latter interval may have had a positive effect on hens fed the highest levels of YSCP.

There was a positive relationship between levels of dietary YSCP and mortality rate (Table 6). Mortality ranged from 1% for hens fed

Table 5. Performance of SCWL hens fed YSCP after supplemental methionine levels were changed, experiment 1[a]

	Levels of Dietary YSCP %			
	.00	6.55	12.90	19.15
Hen-day egg production, %	71.0±1.4[b]	72.4± 1.4	75.3± .9	69.6±2.0
Egg weight, g[c]	62.9± .3	63.3± .4	61.1± .4	60.9± .4
Egg yield, g/hen/day[d]	44.8± .8	45.7± .6	46.0± .6	42.1±1.1
Feed consumed, g/hen/day	100.9±1.4	99.9± .9	99.5± .2	94.8±1.4
Feed efficiency, kg feed/kg eggs[d]	2.3± .04	2.2± .03	2.2± .03	2.3± .06

[a]Means of seven experimental units per treatment for 3, 28-day periods.

[b]Means ± SE.

[c]Significant linear effect of levels of YSCP (P<.01).

[d]Significant quadratic effect of levels of YSCP (P<.01).

Table 6. Effect of YSCP on body weights and mortality[a], experiment 1

	Levels of dietary YSCP %			
	00	6.55	12.90	19.15
Body weight gain (1-7 periods)**	219 ±33[b]	218 ±52	157 ±15	122 ±33
Body weight gain (8-11 periods)	103 ±10	124 ±30	93 ±20	160 ±35
Mortality, %**	1.2± 3	11.3± 7	15.1± 4	27.0± 5
Fatal liver rupture, n/N[c]	1/2	4/9	5/10	10/16

[a]Overall means of eight hens per experimental unit and seven units per treatment.

[b]Means ± SE.

[c]Number of deaths from fatal liver rupture/total number of deaths.

**Significant linear effect of levels of YSCP (P<.01).

the diet containing no YSCP to 27% for hens fed the diet containing 19.15% YSCP. The adverse effect of YSCP on livability was observed throughout the experiment, and extra supplemental methionine did not alter this pattern of treatment effect. Postmortem examination showed that a high proportion of the deaths was the result of fatal liver rupture. The relative incidence of fatal liver rupture was similar for all diet treatments.

There were no differences among dietary treatments with respect to the absolute weights of the liver, heart, proventiculus, kidneys and lungs (Table 7). There was a significant decrease in the weight of the pancreas and an increase in the weight of the empty cecum of hens fed the diets containing 6.55, 12.9, and 19.15 % YSCP. The absolute organ weights were adjusted to percentage of live body weights. The relative organ weights showed a similar trend in terms of dietary treatment effects as seen for the absolute organ weights (Table 8).

Experiment 2

Dietary YSCP had no significant effect on rate of egg production, egg weight, egg yield and feed efficiency, nor was there any significant effect of supplemental Se observed for these criteria (Table 9). Although there was no significant effect of levels of dietary YSCP on feed intake, hens fed the diet containing 18.6% YSCP consumed about 5% less feed than those fed the control diet. Supplementation of diets with Se significantly increased feed consumption across all levels of YSCP. However, no significant interaction between levels of YSCP and levels of Se was observed for this criterion.

Table 7. Effect of YSCP on organ weights of SCWL hens[a], experiment 1

	Levels of YSCP, (%)			
	00	6.55	12.90	19.15
Live weights, g	1700 ±62[b]	1700 ±50	1571 ±49	1587 ±42
Liver, g	24.6± .9	23.2± .8	21.6± .5	23.0± 1
Heart, g	5.5± .1	5.1± .2	5.1± .2	5.0± .2
Pancreas, g**	2.8± .1	2.4± .1	2.1± .1	2.1± .1
Proventiculus, g	4.0± .2	4.0± .1	3.9± .1	3.9± .1
Cecum empty, g**	1.4± .05	1.5± .06	1.5± .04	1.7± .04
Kidneys, g	11.1± .3	10.4± .4	10.4± .3	10.4± .2
Lungs, g	5.6± .2	5.8± .2	5.6± .2	5.7± .2

[a]Overall means of two hens per experimental unit and seven experimental units per treatment.

[b]Means ± SE.

**Significant linear effect of levels of YSCP ($P<.01$).

Table 8. Effects of YSCP on organ weights of SCWL hens[a], g/100 g of body weight, experiment 1

	Levels of YSCP (%)			
	00	6.55	12.90	19.15
Live weights, g	1700 ±62	1700 ±50	1571 ±49	1587 ±42
Liver, g	1.47± .05	1.38± .06	1.38± .04	1.45± .04
Heart, g	0.33± .01	0.31± .01	0.32± .01	0.32± .01
Pancreas, g**	0.16± .01	0.14± .01	0.13± .005	0.13± .006
Proventiculus, g	0.24± .01	0.24± .01	0.25± .004	0.24± .01
Cecum empty, g**	0.08± .003	0.09± .005	0.10± .003	0.10± .005
Kidneys, g	0.66± .02	0.62± .02	0.66± .01	0.66± .01
Lungs, g	0.34± .01	0.34± .01	0.35± .01	0.36± .01

[a]Overall means of two hens per experimental unit and seven experimental units per treatment. All weights are expressed as g/100 g live body weights.

[b]Means ± SE.

**Significant linear effect of level of YSCP ($P<0.01$).

Table 9. Effect of Se and YSCP on the performance of SCWL hens, experiment 2

	Se	Dietary levels of YSCP (%)[a] 00	6.50	12.70	18.60	$\bar{x}$
Hen-day egg production, %	-	80.5	78.1	82.3	82.9	81.0 ±.7
	+	85.2	80.7	85.8	77.7	82.4 ±.8
	$\bar{x}$	82.9 ±1[b]	79.4 ±1	84.1 ±1	80.4 ±1	
Egg weight, g	-	58.3	59.1	58.1	57.4	58.2 ±.3
	+	58.5	59.0	59.0	58.2	58.7 ±.3
	$\bar{x}$	58.4 ±.4	59.0 ±.5	58.5 ±.4	57.8 ±.4	
Feed consumed, g/hen/day	-	98.1	95.0	95.2	94.0	95.6 ±.5
	+	101.4	97.3	100.5	96.3	98.91* ±.6
	$\bar{x}$	99.8 ±.7	96.1 ±.8	97.8 ±.8	95.1 ±.9	
Feed efficiency kg feed/kg egg	-	2.1	2.0	2.0	1.9	2.0 ±.01
	+	2.0	2.0	2.0	2.1	2.0 ±.02
	$\bar{x}$	2.0 ±.02	2.0 ±.02	2.0 ±.02	2.0 ±.02	
Egg yield, g/hen/day	-	46.8	46.0	47.9	47.3	47.0 ±.4
	+	49.7	47.6	50.5	45.1	48.2 ±.4
	$\bar{x}$	48.2 ±.5	46.8 ±.5	49.2 ±.6	46.2 ±.6	

[a]Means of three experimental unit per treatment for 11, 28-day periods.

[b]Means ± SE.

*Significant effect of level of selenium ($P<0.05$).

Body weight gain was not affected significantly by inclusion of YSCP in the diets. Supplementation with Se significantly increased body weight, irrespective of YSCP level (Table 10). This main effect of Se may have been the result of increased feed intake of hens fed the diets containing Se (Table 9).

There was no relationship between levels of dietary YSCP or supplemental Se and rate of mortality (Table 10).

Table 10. Effects of Se and YSCP on body weight and mortality, experiment 2

	Se	Levels of dietary YSCP %[a] 00	6.50	12.70	18.60	$\bar{x}$
Weight gain, g	-	273	276	220	240	252 ±14
	+	300	333	263	356	313* ±14
	$\bar{x}$	286 ±17[b]	305 ±28	241 ±14	298 ±28	
Mortality, %	-	0.00	8.40	16.30	5.60	7.57± .4
	+	5.60	10.50	5.60	3.00	6.30± .3
	$\bar{x}$	2.80± .6	9.45± .5	10.95± .5	4.30± .9	

[a]Means of three experimental unit per treatment for 11, 28-day periods.

[b]Means ± SE.

DISCUSSION

Inclusion of YSCP at dietary levels of 0, 6.55, 12.9 or 19.15% resulted in a decrease in average egg weight, egg yield, feed consumption and body weight gain for the first seven periods in experiment 1. Neither rate of egg production nor feed efficiency was affected by the dietary treatments. It was postulated that the reductions in egg weight, egg yield and weight gain by hens fed YSCP were the result of the decline in feed intake and, consequently, insufficient intake of essential nutrients such as TSAA. The feed efficiency (kg feed/kg egg) data indicated that hens fed YSCP diets utilized feed as efficiently for egg production as hens fed the diet containing only SBM as the main protein source. This also indicated that the reduction in performance of hens fed YSCP diets probably was associated with reduced feed intake. The YSCP tested was in a dry, powdery form and imparted a dusty, fine particle texture to the all-mash diets. Consequently, an adverse effect of YSCP on palatability of diet and on feed intake would be anticipated. Waldroup et al. (1971) and White and Balloun (1977) observed a reduction in feed intake of broiler chicks fed YSCP in all-mash diets. These researchers also found that feeding the diets in pelleted form eliminated the palatability problem.

The results of experiment 2 seem to contradict those of experiment 1 in that the level of dietary YSCP had no effect on feed intake. An examination of the diets used in the two experiments indicates that palatability of the diets containing YSCP was probably a contributing

factor for this difference between experiments. In experiment 1, the level of supplemental fat decreased markedly as the dietary YSCP level increased. The level of supplemental fat used in experiment 2, however, exceeded 2% in all diets. Fats are known to improve the palatability of fine-textured diets that are fed in mash form (Scott et al., 1976). Possibly, the higher levels of dietary fat used in experiment 2 as compared with experiment 1 alleviated the adverse effect of YSCP on palatability and, therefore, no main effect of YSCP on feed intake was observed in experiment 2.

The greatest disagreement between experiments 1 and 2 occurred with mortality. The significant linear effect of dietary YSCP whereby mortality was increased in experiment 1 was not observed in experiment 2. At the end of experiment 1, it was postulated that hens fed the YSCP-containing diets may have been receiving inadequate Se. This seemed reasonable because Ikumo et al. (1978) and Succi et al. (1980) reported that YSCP grown on a methanol substrate was deficient in Se, and broiler chicks fed this type of YSCP developed signs of Se deficiency. In experiment 1, the calculated Se concentrations of diets containing 0, 6.55, 12.9 and 19.15% YSCP were .052, .051, .045 and .041 ppm, respectively. These levels were less than the dietary level of .1 ppm recommended by NRC (1977). Therefore, Se supplementation of YSCP diets was evaluated in experiment 2.

The results showed that neither supplemental Se nor levels of YSCP affected mortality significantly. It must be emphasized, however, that the levels of supplemental methionine used in the YSCP diets of experiment

2 were higher than those used during periods 1 through 7 or 8 through 11 of experiment 1. The possibility exists that the high mortality observed in experiment 1 may have been related to inadequate methionine and/or TSAA intake by hens fed YSCP containing diets. TSAA intakes were particularly low during the first seven periods of experiment 1, and the physiological stresses of egg production would have been greatest during this interval. Inadequate TSAA consumption by laying hens has been shown to result in the accumulation of excess fat in the liver and fatal liver rupture has been associated with fatty liver (Jensen, 1979). Although the proportion of deaths occurring due to fatal liver hemorrhage was the same across all dietary treatments, the number of fatal liver ruptures increased with increments of dietary YSCP fed in experiment 1.

Hens of experiment 2 were fed relatively high levels of TSAA throughout the trial. Because of the experimental design, however, it was not possible to determine whether or not this extra TSAA intake per se alleviated the effect of YSCP on mortality.

An increase in the empty cecal weight was observed for hens fed YSCP diets in experiment 1. This increase in cecal weight may have been related to the presence of undigested yeast carbohydrates in the ingesta of hens fed the YSCP diets (Gaillard and Van Weerden, 1976). These carbohydrates may have fermented in the cecum and caused its enlargement (Leegwater et al., 1974). The effect of an increase in cecal size on nutritional requirements of the animals is not clear. However, an increase in cecal size is usually associated with an increase in microflora in the

cecum and may result in the proliferation of detrimental as well as beneficial microorganisms within the intestine. It has been reported that rye in the diets of chicks altered the gut microflora and reduced chick growth (Wagner and Thomas, 1978). Additional research showed that these effects of rye were alleviated by supplementing the diets with antibiotics or methionine (Patel and McGinnis, 1980). It is possible that the increase in cecal size noted in experiment 1 may have resulted in an increased requirement of TSAA via changes in gut microflora.

In general, rate of egg production, egg weight and egg yield of hens fed diets containing YSCP and supplemented with methionine were equal to those fed the control diet. In both experiments, efficiency of feed utilization was unaffected by inclusion of YSCP in the diets. These results suggest that the protein of YSCP tested here is comparable to that of SBM, provided that the YSCP protein is adequately supplemented with DL methionine.

Two main concerns about the use of YSCP in laying diets remain. These are the apparent adverse effect of increments of YSCP on feed intake and mortality observed in experiment 1. Seemingly, the inclusion of at least 2% supplemental fat and additional supplemental methionine alleviated the effects of YSCP on feed intake and mortality in experiment 2. More research is needed to obtain definitive information on these aspects of YSCP use in diets for laying hens.

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SECTION III. NUTRITIONAL EVALUATION OF YEAST SINGLE-CELL PROTEIN AS A SOURCE OF AMINO ACIDS FOR CHICKENS OVER TWO GENERATIONS

NUTRITIONAL EVALUATION OF YEAST SINGLE-CELL PROTEIN AS A SOURCE OF AMINO ACIDS FOR CHICKENS OVER TWO GENERATIONS

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ABSTRACT

An experiment was conducted to evaluate YSCP as a source of amino acids for Single Comb White Leghorn chickens over two generations. The experiment was of a 2 x 4 factorial arrangement with two generations (FO and Fl) and four dietary levels of YSCP. The four dietary levels of YSCP were obtained by substituting YSCP for 0, 33, 66 or 100% of the dietary SBM protein. Each generation was divided into three phases: starter, grower and breeder. Replacing 100% of the dietary SBM protein with the YSCP during the starter phase significantly reduced average daily gain (ADG) and feed intake. However, body growth during the grower and the breeder phases was not affected by inclusion of YSCP in the diets. Nor was there any effect of dietary YSCP on efficiency of feed utilization during the starter or grower phases. Inclusion of YSCP in the diets during the breeder phase did not significantly affect rate of egg production, egg weight, egg yield, efficiency of conversion of feed into eggs, hatchability of fertile eggs, hatchability of all eggs incubated and distribution of embryonic death. However, substituting YSCP for 66 or 100% of the dietary SBM protein significantly reduced fertility. Growth and reproduction data of males were not recorded during breeder phase of the FO generation. In the Fl generation, inclusion of YSCP in the diets had no significant effect on body weight, feed consumption, percentage of males producing semen or semen volume per ejaculate. In this experiment, no physical abnormalities in embryos or in live chicks were observed that could be attributed to dietary YSCP. Mortality

was low throughout the experiment and was not related to the dietary treatments.

INTRODUCTION

Conventional methods of food production such as farming and animal husbandry have had increasing difficulty in keeping pace with the ever growing demand for protein. Among the alternatives sought to overcome the protein shortage, yeast single-cell protein (YSCP) has received much attention from animal scientists. Yeast products have received this attention because they are generally accepted as food sources for humans, and because yeast has been grown successfully on several substrates on a pilot plant scale (Laskin, 1977).

YSCP grown on n-paraffin, n-alkane or gas-oil has been used successfully to replace 50 to 100% of a conventional protein source in diets of replacement pullets, laying hens, and reproducing chickens (Waldroup and Hazen, 1975; Shannon et al., 1976; VanderWal, 1976; Jackson and Kirkpatrick, 1978; and Yoshida, 1979).

Similarly, the protein (YSCP) of a specific yeast grown on methanol has been evaluated as a source of amino acids for rats (Ashraf et al., 1981; and Ashraf and Vetter, 1981), for broiler chicks (White and Balloun, 1977), and for pigs (Tegbe and Zimmerman, 1977; and Slagle and Zimmerman, 1979). These studies have shown that YSCP can be used successfully in diets of these animals to support growth and reproductive performance, provided that the YSCP diets are adequately supplemented with DL methionine. Recently, the process for producing the particular YSCP was modified. Sell et al. (1981) tested the "new" YSCP and reported that this protein source could be substituted for 75% of the dietary soybean meal (SBM)

protein without an adverse effect on growth of broiler chicks provided that the YSCP diets were pelleted, or contained 2 to 2.5% supplemental fat, and were supplemented with adequate levels of methionine and arginine. Ashraf and Sell (1981a) evaluated this same YSCP as a source of amino acids for laying hens in an experiment in which YSCP was used to replace 100% of the dietary SBM protein. The results showed that the YSCP diets supplemented with methionine supported satisfactory laying hen performance.

The objective of the experiment described herein was to obtain additional information about the nutritional characteristics of the "new" YSCP when this protein source was used in diets of chickens for two successive generations.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was a 2 x 4 factorial arrangement of two generations (FO and F1) and four levels of dietary YSCP. The four levels of YSCP were obtained by substituting YSCP for 0, 33, 66 or 100% of the dietary protein supplied by soybean meal (SBM). Each generation was divided into starter (0-6 weeks), grower (7-18 weeks) and breeder (19-42 weeks) phases. The parent generation (FO) consisted of 500 unsexed, day-old Single Comb White Leghorn (SCWL) chicks from the closed flock at Iowa State University. The chicks were allotted at random to 20 pens located in electrically-heated batteries. Thus, each pen of 25 chicks constituted an experimental unit, and five experimental units were assigned randomly to each of the four dietary treatments within each generation. The chicks were fed starter phase diets and were provided with continuous light from one day to six weeks of age.

At the end of the starter phase, chicks were debeaked and transferred to floor pens equipped with raised wood-slat floors. The chickens were kept in these pens from 6 to 18 weeks of age, during which grower phase diets were fed and eight hours of light per 24 hour days were provided. During the starter and grower phases, body weight and feed consumption data were recorded at 0, 6, and 18 weeks. At the end of the grower phase, 40 pullets and five roosters were selected randomly from each dietary treatment. The 40 pullets were allocated randomly to 20 cages.

The floor dimensions of each cage were 25.4 cm wide x 40.6 cm deep. A group of four cages was designated as an experimental unit; thus, each unit contained eight pullets and there were five experimental units per dietary treatment.

The roosters were not dubbed; therefore, they could not be housed in normal laying hen cages. Instead, the roosters were kept in a separate building. During the breeder phase, pullets were fed breeder phase diets and roosters were fed grower phase diets. The chickens were provided with 14 hours of light per 24 hour day from 18 to 42 weeks of age. The F0 generation was terminated at the age of 42 weeks.

Four weeks after the hens reached 50% egg production, each hen was artifically inseminated with pooled semen from males fed the same level of dietary YSCP. The hens were inseminated weekly for twelve weeks. All eggs produced beginning forty-eight hours after the first insemination were collected and stored in a refrigerated storeroom to await incubation. The eggs were held no longer than seven days before incubation after each of the first eleven collections, but were held ten days for the last collection. Incubation was done in a Jamesway 252B incubater. Separate fertility and hatchability data were recorded for each experimental unit. On the 18th day of incubation, the eggs were candled and placed in the hatching trays. On the 22nd day of incubation, the chicks were removed from the incubator and live chicks were examined for physical abnormalities. The infertile and unhatched eggs were broken to determine true fertility. The clear eggs with no sign of embryos were recorded as infertile eggs.

The dead embryos and unhatched chicks were examined to determine their approximate age at death.

After the last hatch (12th), the live chicks were grouped according to dietary treatments, and were sexed. The males were dubbed. Sixty-five chicks of each sex were selected randomly from the hatch of each dietary treatment to carry on the second generation phase (F1) of the experiment. The chicks were divided into five groups of 26 each with males and females represented equally. The chicks were weighed and assigned randomly to five experimental units per dietary treatment. All F1 chicks were fed the same diet treatments as their parents had received.

During the breeder phase, body weights were recorded at the start of the breeder phase (18 weeks), at the start of artificial insemination (30 weeks), and at the end of the generation (42 weeks). Feed consumption was recorded each 28-days or when the phase ended. Egg production and mortality were recorded daily. Postmortem examination to determine the cause of death was done by a veterinarian at the Iowa State University Veterinary College.

Diets

The chickens were provided all-mash diets and water ad libitum throughout the experiment. The diets were formulated to provide 2900, 2930, and 2900 kcal of metabolizable energy (ME)/kg and to provide 20, 15, and 16.4% crude protein during starter, grower and breeder phases, respectively.

Four diets were tested in each phase to evaluate YSCP as a source of amino acids for SCWL chickens. The control diet was composed primarily of corn and SBM (Tables 1 and 2). Three additional test diets were obtained by substituting YSCP for 33, 66 or 100% of SBM protein. Consequently, YSCP constituted 0, 6.5, 13, and 19.6% of the diets in the starter phase; 0, 4.7, 8.0, and 11.6% in the grower phase; and 0, 6, 11.3, and 16.5% in the breeder phase (Table 1). The ME of the YSCP used in the diet formulation was 2900 kcal/kg. The chemical composition of YSCP was 61.0% crude protein, 55% true protein, .64% methionine, .35% cystine, .1% calcium, and 2.8% phosphorus. The true protein value of YSCP was used for diet formulation. The YSCP also was used as a phosphorus source in the diets (Tables 1 and 2). This was done because Ashraf and Sell (1981b) had shown that the phosphorus from the YSCP was 70% as available as the phosphorus from a reference phosphorus source, tri-calcium phosphate.

The diets containing YSCP in the breeder phase of the F0 generation and subsequent diets during the starter, grower and breeder phases of the F1 generation were supplemented with higher levels of DL methionine than those recommended by NRC (1977). This resulted in total sulfur amino acid (TSAA) levels of .61, .63, .66 and .68% for the diets containing 0, 33, 66 or 100% YSCP protein, respectively, during breeder phase of generation F0. The TSAA contents of diets containing 0, 33, 66 or 100% YSCP protein in generation F1 were .66, .72, .78 and .84% of the diets in the starter phase; .51, .54, .59, and .61% in the grower phase; and

Table 1. Composition of diets used in F0 generation

	Chick starter diets			
	Levels of YSCP %			
	00	6.5	13.0	19.6
Corn	25.28	25.96	26.96	27.80
Oats	37.92	38.94	40.44	41.70
SBM	22.20	14.85	7.39	-
YSCP	-	6.51	13.00	19.60
Corn gluten meal	3.50	3.50	3.00	2.50
Alfalfa meal	2.50	2.50	2.50	2.50
Fat	4.50	3.85	3.00	2.40
Limestone	1.19	1.66	2.13	2.60
Dicalcium phosphate	2.11	1.41	0.71	-
Vitamin premix[a]	0.50	0.50	0.50	0.5
Mineral premix[b]	0.30	0.30	0.30	0.3
DL methionine	-	0.04	0.07	0.1
	100.00	100.00	100.00	100.00
Crude protein, %	20.0	20.2	20.2	20.1
ME kcal/kg	2904	2909	2903	2906
Methionine, %	0.35	0.38	0.41	0.43
TSAA, %	0.68	0.68	0.68	0.67
Total phosphorus, %	0.76	0.76	0.76	0.76
Calcium, %	1.13	1.13	1.13	1.13

[a]Supplied the following per kg of diet: Vitamin A, 7500 IU; Vitamin D_3, 1000 IU; Vitamin E, 10 IU; Vitamin K, 2.2 mg; Vitamin B_{12}, 10 µg; riboflavin, 5 mg; choline, 450 mg; pantothenate, 10 mg; niacin, 25 mg; ethoxyquin, 100 mg.

[b]Supplied the following per kg of diet: Cu, 6 mg; Fe, 37 mg; Mn, 70 mg; Se, .1 mg; Zn, 40 mg.

Table 1 continued.

	Grower diets			
	Levels of YSCP %			
	00	4.1	8.0	11.6
Corn	46.95	47.85	48.78	49.71
Oats	31.40	32.00	32.61	33.27
SBM	14.00	9.24	4.50	-
YSCP	-	4.07	8.00	11.60
Alfalfa meal	2.50	2.50	2.50	2.50
Fat	1.80	1.20	0.60	-
Limestone	0.95	1.24	1.53	1.80
Dicalcium phosphate	1.44	1.00	0.47	0.10
Vitamin premix[c]	0.50	0.50	0.50	0.50
Mineral premix[d]	0.30	0.30	0.30	0.30
Tinostat[e]	0.15	0.15	0.15	0.15
DL methionine	0.02	0.04	0.06	0.07
	100.00	100.00	100.00	100.00
Protein %	15.10	15.21	15.24	15.22
ME kcal/kg	2937	2936	2935	2930
Methionine, %	0.27	0.29	0.31	0.31
TSAA, %	0.52	0.52	0.52	0.50
Total phosphorus, %	0.57	0.58	0.57	0.58
Ca, %	0.77	0.78	0.77	0.79

[c]Supplied the following per kg of diet: Vitamin A, 7500 IU; Vitamin D_3, 1000 IU; Vitamin E, 10 IU; Vitamin K, 2 mg; Vitamin B_{12}, 10 µg; riboflavin, 5 mg; choline, 250 mg; pantothenate, 5 mg.

[d]Supplied the following per kg of diet: Cu, 6 mg; Fe, 37 mg; Mn, 70 mg; Se, .1 mg; Zn, 40 mg.

[e]Tinostat, Salsbury Laboratories, Inc., Charles City, IA.

Table 1 continued.

	Breeder diets			
	Levels of YSCP %			
	00	6.0	11.3	16.5
Corn	52.90	51.23	49.17	46.67
Oats	13.20	17.11	21.08	25.18
SBM	20.70	13.21	6.5	-
YSCP	-	6.0	11.3	16.49
Fat	3.00	2.6	2.3	2.20
Limestone	7.30	7.6	8.0	8.40
Dicalcium phosphate	2.03	1.3	0.65	-
Vitamin premix[f]	0.50	0.5	0.5	0.50
Mineral premix[g]	0.30	0.3	0.3	0.30
DL methionine	0.07	0.14	0.2	0.26
	100.00	100.00	100.00	100.00
Protein, %	16.43	16.44	16.44	16.45
ME kcal/kg	2905	2904	2903	2906
Methionine, %	0.35	0.40	0.45	0.50
TSAA, %	0.61	0.63	0.66	0.68
Total phosphorus, %	0.69	0.68	0.67	0.66
Ca, %	3.3	3.2	3.2	3.2

[f]Supplied the following per kg of diet: Vitamin A, 8000 IU; Vitamin D_3, 2400 IU, Vitamin B_{12}, 5 µg; riboflavin, 6.6 mg; pantothenate, 6.6 mg; niacin, 22 mg; ethoxyquin, 11 mg.

[g]Supplied the following per kg of diet: Mn, 20 mg; iodized salt, 2.997 g; Se, .1 mg.

Table 2. Composition of diets used in F1 generation

	Starter diets			
	Levels of YSCP %			
	00	8.3	16.45	24.1
Corn	42.70	43.80	45.50	46.80
Oats	18.30	18.90	19.49	20.10
SBM	29.10	19.30	9.30	-
YSCP	-	8.30	16.45	24.10
Alfalfa meal	2.50	2.50	2.50	2.50
Fat	2.70	2.70	2.60	2.60
Limestone	1.07	1.71	2.20	2.72
Dicalcium phosphate	2.80	1.84	0.90	-
Vitamin premix[a]	0.50	0.50	0.50	0.50
Mineral premix[b]	0.30	0.30	0.30	0.30
DL methionine	0.03	0.15	0.27	0.38
	100.00	100.00	100.00	100.00
Protein %	20.0	20.0	20.0	20.0
ME kcal/kg	2906	2902	2903	2905
Methionine, %	0.35	0.45	0.56	0.66
TSAA, %	0.66	0.72	0.78	0.84
Total phosphorus, %	0.87	0.87	0.87	0.86
Calcium, %	1.13	1.15	1.12	1.13

[a]Supplied the following per kg of diet: Vitamin A, 7500 IU; Vitamin D_3, 1000 IU; Vitamin E, 10 IU; Vitamin K, 2.2 mg; Vitamin B_{12}, 10 µg; riboflavin, 5 mg; choline, 450 mg; pantothenate, 10 mg; niacin, 25 25 mg; ethoxyquin, 100 mg.

[b]Supplied the following per kg of diet: Cu, 6 mg; Fe, 37 mg; Mn, 70 mg; Se, .1 mg; Zn, 40 mg.

Table 2 continued.

	Grower diets			
	Levels of YSCP %			
	00	4.6	9.1	13.1
Corn	45.95	46.66	47.40	48.00
Oats	30.90	31.20	31.70	32.00
SBM	15.90	10.40	4.90	-
YSCP	-	4.60	9.10	13.10
Alfalfa meal	2.50	2.50	2.50	2.50
Fat	1.50	1.50	1.40	1.40
Limestone	0.83	1.14	1.43	1.80
Dicalcium phosphate	1.46	0.97	0.47	-
Vitamin premix[c]	0.50	0.50	0.50	0.50
Mineral premix[d]	0.30	0.30	0.30	0.30
Tinostat[e]	0.15	0.15	0.15	0.15
DL methionine	0.02	0.08	0.17	0.23
	100.00	100.00	100.00	100.00
Protein, %	15.0	15.0	15.0	15.0
ME kcal/kg	2930	2929	2922	2923
Methionine, %	0.27	0.32	0.40	0.44
TSAA, %	0.51	0.54	0.59	0.61
Total phosphorus, %	0.58	0.59	0.59	0.59
Calcium, %	0.73	0.74	0.73	0.74

[c]Supplied the following per kg of diet: Vitamin A, 7500 IU; Vitamin D_3, 1000 IU; Vitamin E, 10 IU; Vitamin K, 2 mg; Vitamin B_{12}, 10 mg; riboflavin, 5 mg; choline, 250 mg; pantothenate, 5 mg.

[d]Supplied the following per kg of diet: Cu, 6 mg; Fe, 37 mg; Mn, 70 mg; Se, .1 mg; Zn, 40 mg.

[e]Tinostate, Salsbury Laboratories, Inc., Charles City, IA.

Table 2 continued.

	Breeder diets			
	Levels of YSCP %			
	00	6.50	12.70	18.60
Corn, %	64.55	66.	68.10	69.50
SBM	22.50	14.80	7.10	-
YSCP	0.0	6.40	12.70	18.60
Fat	2.50	2.50	2.15	2.16
Limestone	7.20	7.76	8.23	8.60
Dicalcium phosphate	2.38	1.52	0.67	0.00
Vitamin premix[f]	0.50	0.50	0.50	0.50
Mineral premix[g]	0.30	0.30	0.30	0.30
DL methionine	0.07	0.16	0.25	0.34
	100.00	100.00	100.00	100.00
Protein, %	16.65	16.67	16.67	16.65
ME kcal/kg	2965	2966	2962	2968
Methionine, %	0.36	0.44	0.51	0.58
Cystine, %	0.26	0.23	0.20	0.17
TSAA, %	0.62	0.67	0.71	0.75
Total phosphorus, %	0.76	0.74	0.71	0.71
Calcium, %	3.31	3.33	3.31	3.30

[f]Supplied the following per kg of diet: Vitamin A, 8000 IU; Vitamin D_3, 2400 IU, Vitamin B_{12}, 5 µg; riboflavin, 66 mg; pantothenate, 6.6 mg; niacin, 22 mg; ethoxyquin, 11 mg.

[g]Supplied the following per kg of diet: Mn, 20 mg; iodized salt, 2.997 g; Se, .1 mg.

.62, .67, .71 and .75% in the breeder phase, respectively (Tables 1 and 2). These TSAA levels were used because Ashraf and Sell (1981a) had shown that performance of laying hens was improved by feeding YSCP diets containing a higher level of supplemental TSAA than that recommended by NRC (1977).

The F1 generation was reared in the same manner as was the F0 generation except for the following changes: 1) the ME value of YSCP used for diet formulation was reduced from 2900 ME kcal/kg to 2200 ME kcal/kg. This was done because Bales (1980) had determined experimentally that the ME value of the YSCP for chickens was 2200 kcal/kg. 2) The chicks were placed on 12 hours of light during the growing phase instead of 8 hours. This was necessary because laying hens kept in the same house as used for growing the pullets required 12 hours of light daily. 3) Twelve males per dietary treatment were selected for the breeder phase instead of five. These males were allotted randomly to three experimental units with each unit containing four males.

Growth and reproductive parameters were subjected to statistical analyses as described by Steel and Torrie (1960).

RESULTS

Starter Phase

Average daily gain, feed intake and feed efficiency data are summarized in Table 3. Analyses of variance of these data are shown in Table 4. Substituting YSCP for 33 or 66% of the dietary SBM protein had no significant effect on average daily gain to 6 weeks of age. However, replacing 100% of the SBM protein with the YSCP significantly ($P<.01$) reduced ADG as compared with the ADG of chicks fed the lower levels of YSCP. The chicks fed the 100% YSCP diet gained an average of 1 g per day less than those fed the control diet.

A significant ($P\leq.01$) main effect of generation was observed in that the F0 chicks had a significantly lower ADG than did those of the F1 generation. No significant interaction between generation and dietary levels of YSCP was observed.

Although levels of dietary YSCP had no significant ($P>.10$) main effect on feed intake, partitioning the sum of square into linear and quadratic trends showed a significant ($P\leq.05$) linear effect of dietary YSCP on this parameter. Increasing levels of dietary YSCP decreased feed intake.

A significant difference between generations F0 and F1 was observed in that chicks of the F0 generation consumed significantly less feed than did the F1 generation irrespective of dietary levels of YSCP.

A significant ($P\leq.01$) main effect of dietary YSCP and of generation was observed for feed efficiency. Also, a significant interaction between

dietary YSCP and generation was observed for this criterion. This interaction may have been caused by the inconsistent trends in feed efficiency observed in generation F0 as compared with generation F1. Replacing 100% of the SBM protein with the YSCP increased the feed required per unit of gain in the F0 generation, whereas in the F1 generation, YSCP did not affect feed efficiency.

In general, chicks of the F1 generation utilized feed more efficiently than chicks of the F0 generation and this resulted in a significant main effect of generation.

Grower Phase

Body weights, feed consumption and feed efficiency data are shown in Table 3. Analyses of variance for these data are presented in Table 4. The data obtained in the grower phase of the experiment (6 to 18 weeks of age) were different from those obtained in the starter phase. Inclusion of YSCP in the diets had no significant main effect on the body weight of pullets or roosters. A significant ($P \leq .01$) difference between F0 and F1 generations was observed. Chicks of the F0 generation did not gain as much weight as chicks of the F1 generation.

Significant main effects of both dietary YSCP ($P \leq .05$) and generation ($P \leq .01$) were observed for feed intake. Feed intake by chicks of the F1 generation decreased notably as dietary YSCP replaced 33 or 66% of the protein of SBM. No further decrease in feed intake occurred with the 100% replacement of SBM. Chicks of generation F0 consistently consumed less feed from 6 to 18 weeks of age than those of generation F1,

Table 3. Effect of YSCP on growth of SCWL chickens[a]

	Generation	Replacement of SBM protein, YSCP %				
		00	33	66	100	$\bar{x}$
Six weeks of age						
Daily gain, g	F0	7.8	8.1	8.0	6.8	7.7± .15
	F1	9.5	9.2	9.0	8.5	9.1±*.13
	$\bar{x}$*	8.6±.29[b]	8.6±.22	8.5±.24	7.7±.35	
Feed consumption, g/chick/day	F0	22.8	21.4	22.2	22.1	22.1± .27
	F1	24.3	24.5	23.9	22.6	23.8±*.26
	$\bar{x}$*	23.6±.47	22.9±.57	23.0±.42	22.3±.33	
Feed, g/g gain[c]	F0*	2.9	2.6	2.9	3.2	2.9± .05
	F1	2.6	2.6	2.6	2.6	2.6± .02
	$\bar{x}$*	2.8±.07	2.6±.03	2.8±.05	2.9±.1	
Eighteen weeks of age						
Hens/ body wt., g	F0	762	768	777	777	771±8
	F1	968	962	970	974	968±9
	$\bar{x}$	865±38	865±34	873±34	876±35	
Rooster body wt., g	F0	1305	1234	1238	1218	1249 ±13
	F1	1386	1410	1366	1350	1378* ±12
	$\bar{x}$	1345±19	1322±31	1302±31	1284±25	
Feed consumption[c], g/chick/day	F0	59.8	57.1	57.6	58.9	58.3± .5
	F1*	75.2	74.1	71.3	71.8	73.1±*.5
	$\bar{x}$*	67.5±2.6	65.6±2.8	64.4±2.5	65.3±2.2	

	F0	5.6	5.7	5.8	5.7	5.7± .06
Feed, g/	F1	6.3	6.1	6.0	6.0	6.1±* .05
gain, g	x̄	5.9±.12	5.9±.07	5.9±.08	5.9±.12	
	F0	4/125	1/125	4/124	3/125	12/500
Mortality,	F1	6/130	9/130	4/130	7/130	26/520
n/n		10/255	10/255	8/255	10/255	

[a]Average for five experimental units of 25 chickens each for F0 generation and 26 chickens for the F1 generation.

[b]Means ± SE.

[c]Interaction between diets and generations was significant.

*Main effects of levels of YSCP or generations were significant (P<.05).

Table 4. Analyses of variance of SCWL chickens fed dietary YSCP over two generations

Source of variation	df	Mean squares						
		Six week			Eighteen week			
		ADG	Feed intake	Feed efficiency	Pullet body wt.	Rooster body wt.	Feed intake	Feed efficiency
Generation (G)	1	19.1**	28.8**	1.0**	389075.6**	166152.1**	2178.4**	1.44**
DIET (D)	3	2.2**	2.5	0.14**	312.8	7005.7	16.8*	0.02
Linear	1	4.7**	6.5*	0.22**	820.1	20930.6	30.3**	0.07
Quadratic	1	1.7	0.01	0.18**	13.2	84.1	19.2*	--
G x D	3	0.3	3.1	0.16**	86.5	3816.3	25.2**	0.09
Residual	32	0.3	1.1	0.01	1799.2	2842.9	4.4	0.06
Total	39							

*($P \leq .05$).
**($P \leq .01$).

irrespective of diet. A significant YSCP level x generation interaction reflected the inconsistent influence of dietary YSCP on feed intake by each generation.

Mean feed efficiency values for the grower phase were not significantly affected by dietary YSCP level. However, a significant main effect of generation was observed for feed efficiency whereby the chicks of the F0 generation utilized feed more efficiently than did the chicks of the F1 generation.

During starter and grower phases mortality was low and was not related to dietary YSCP or generation.

Breeder Phase

Data recorded during the breeder phase are presented in Table 5 and the analyses of variance for these data are shown in Table 6. Age at which hens reached 50% egg production was not significantly affected by dietary YSCP nor was there any effect of generation. However, on an average, the hens fed a 100% YSCP diet reached 50% egg production three days later than those fed the control diet.

Similarly, no main effect of dietary YSCP or generation was observed for rate of egg production. Although no main effect of dietary levels of YSCP was observed for egg weight, there was a significant ($P \leq .01$) main effect of generation on egg weight. Hens of the F0 generation laid smaller eggs than did the hens of the F1 generation. No main effect of dietary YSCP or generation was observed for egg yield.

Table 5. Effect of YSCP on performance of SCWL hens[a]

	Generation	Replacement of SBM protein, YSCP %				
		00	33	66	100	$\bar{x}$
Age at 50% production, days	F0	170	166	169	168	168±1
	F1	164	172	169	173	169±2
	$\bar{x}$	167±2[b]	169±2	169±2	170±2	
Hen-day egg production, %	F0	74.6	75.2	77.9	79.1	76.7±.9
	F1	74.6	75.0	81.0	74.8	76.3±1.0
	$\bar{x}$	74.6±1.2	75.1±1.4	79.4±.9	76.9±1.6	
Egg weight, g	F0	53.9	53.9	52.9	53.5	53.6±.4
	F1	57.4	56.1	54.5	55.8	56.0±.3
	$\bar{x}$	55.6±.6	55.0±.6	53.7±.5	54.7±.5	
Egg yield[c] g/hen daily	F0	40.2	40.5	41.3	42.3	41.1±.5
	F1	42.9	42.1	44.1	41.8	42.7±.6
	$\bar{x}$	41.5±.8	41.3±.9	42.7±.6	42.1±	
Feed consumption[d], g/hen daily	F0	93.5	98.4	96.5	101.3	97.4±1
	F1*	95.9	101.0	96.4	93.4	96.7±1
	$\bar{x}$	94.7±1.5	99.7±1.2	96.4±1.2	97.4±1.6	
Feed efficiency, kg feed/kg egg	F0	2.33	2.44	2.34	2.41	2.38±.02
	F1	2.24	2.43	2.18	2.26	2.28*±.03
	$\bar{x}$	2.28±.03	2.43±.04	2.26±.03	2.33±.04	
Body weight gain, g	F0	365	377	347	341	357±12
	F1	372	452	428	436	422*±15
	$\bar{x}$	368±26	414±22	387±18	388±19	

	F0	1/40	2/40	0/40	0/40	3/160
Mortality, n/n	F1	3/40	2/40	0/40	0/40	5/160
	$\bar{x}$	4/80	4/80	0/80	0/80	

[a]Average for five experimental units of eight hens each for the breeder phase of three, 28-day periods.

[b]Means ± SE.

[c]Egg yield was calculated by multiplying average egg weight times the number of eggs produced and dividing this product by the number of hen days.

[d]Interaction between diets and generation was significant.

*Main effects of levels of YSCP or generation were significant ($P<.05$).

Table 6. Analyses of variance of SCWL hens fed dietary YSCP over two generations

Source of variation	dF		Egg prod.	Egg wt.	Egg yield	Feed intake	Feed efficiency	Age 50% prod.	Body wt. gain
Generation (G)	1		3.5	169.7**	81.0	15.4	0.31**	16.9	41280.6**
DIET (D)	3		141.0	18.7	11.6	130.4	0.17*	16.2	3538.5
Linear	1		188.3	25.2	13.1	34.9	0.002	40.5	554.4
Quadratic	1		67.4	18.8	1.3	128.6	0.05	0.0	4950.6
G x D	3		68.4	4.9	17.3	182.7*	0.04	77.1	3857.2
Pen (G x D)	32		84.2	8.3	29.0	49.0	0.06	47.3	3610.8
Period (P)[a]	2	(1)[b]	585.0**	219.4**	426.9**	1640.8**	0.27**		
P x G	2	(1)	196.3**	20.2**	22.5	63.1*	0.05		
P x D	6	(3)	24.4	1.4	10.3	9.3	0.02		
P x G x D	6	(3)	37.3	0.6	14.5	17.8	0.03		
Residual	64	(32)	19.4	0.9	6.0	16.2	0.02		
Total	119								

[a]Each period was 28 days in length.

[b]Geisser-Greenhouse procedure for conservative tests was used to find probability of greater F value (Gill, 1978).

*($P \leq .05$).

**($P \leq .01$).

Although no significant main effect of either dietary YSCP or generation were observed for feed intake, there was a significant interaction between dietary YSCP and generation observed for feed intake. This interaction may have been caused by the inconsistent trends in feed intake observed in generation F0 as compared with generation F1. Substituting YSCP for 33, 66 or 100% of the dietary SBM increased the feed intake of hens of the F0 generation, whereas replacing SBM protein with the YSCP at 33 or 100% decreased the feed intake of hens of the F1 generation.

There were significant main effects of YSCP ($P<.05$) and generations ($P\leq.01$) on feed efficiency. In the case of dietary YSCP effect, it did not appear to be related to the levels of dietary YSCP. Hens of the F0 generation were less efficient in converting feed into eggs than were hens of the F0 generation.

Increasing levels of dietary YSCP in the breeder phase had no significant main effect on body weight gain. Also, no significant main effect of generation on body weight gain was observed.

During the breeder phase, mortality was low and was not related to the dietary treatments or the generations.

The data for fertility and hatchability of fertile eggs and hatchability of eggs incubated are summarized in Table 7. Analyses of variance for these data are shown in Table 8. A significant ($P<.01$) main effect of dietary YSCP was observed for fertility in that hens fed 66 or 100% YSCP diet laid significantly fewer fertile eggs than did those fed the

Table 7. Effect of YSCP on performance of SCWL reproducing chickens[a]

	Gene	Percent protein from YSCP 00	33	66	100	$\bar{x}$
No. of eggs incubated	F0	2401	2328	2588	2654	9971
	F1	2306	2382	2521	2462	9671
	$\bar{x}$	4707	4710	5109	5116	
Fertility %	F0*	93.03	94.66	89.75	89.46	91.72
	F1	92.81	91.17	89.44	89.56	90.75
	$\bar{x}$*	92.92±.6[b]	92.91±.6	89.59±.7	89.51±.6	
Hatch %	F0	93.83	94.49	95.08	93.74	94.28
	F1	86.28	89.15	89.59	89.09	88.53*
		90.05±.6	91.82±.5	92.33±.5	91.41±.7	
Net hatch %	F0	87.24	89.60	85.44	84.56	86.71
	F1	80.14	81.21	80.01	79.81	80.29*
		83.69±.8	85.41±.8	82.73±.7	82.19±.9	

[a]Average for five experimental units of eight hens for the breeder phase of 12, 7-day periods.

[b]Means ± SE.

*Significant effect of levels of YSCP or generations (P<.05).

Table 8. Analysis of variance of SCWL chickens fed dietary YSCP over two generations

Source of variation	dF		Mean Squares		
			Fertility	Hatchability	Net hatch
Generation (G)	1		114.7	3982.0**	4947.2**
DIET (D)	3		454.1**	114.8	240.1
Linear	1		1104.6**	127.1	310.3
Quadratic	1		0.2	217.3	153.0
G x D	3		85.3	46.6	81.2
P (G x D)	32		75.5	128.3	215.4
Period[a] (P)	11	(1)[b]	386.4**	50.4	238.9**
P x G	11	(1)	141.8*	48.6	152.5
P x D	33	(3)	40.3	32.5	70.5
P x G x D	33	(3)	35.8	26.5	42.4
Residual	352	(32)	30.7	25.5	46.4
Total	479				

[a]Each period was 7 days in length.

[b]Geisser-Greehnouse procedure for conservative tests was used to find probability of greater F value (Gill, 1978).

*($P \leq .05$).

**($P \leq .01$).

control or the 33% YSCP diets. Consequently, the percent fertility of all eggs produced was 3% less for hens fed diets containing 66 or 100% YSCP than for hens fed the other diet treatments. No main effect of generation on fertility of eggs was observed.

Hatchability of fertile eggs and hatchability of all eggs incubated (net hatch) were not affected significantly by dietary treatments. However, for both these criteria, a significant main effect of generation was observed in which hatchability of fertile eggs and net hatch were higher during the F1 generation.

Levels of dietary YSCP did not significantly affect the distribution of embryonic deaths in the age groups 1 to 7 or 8 to 14 days. However, for the age 15 to 21 days, there were main effects of dietary treatments ($P \leq .01$). In this age group, embryonic deaths were higher for hens fed the control diet than for those fed the YSCP diets. All these three age groups had a significant ($P \leq .01$) main effect of generation in that for age group 1 to 7 or 15 to 22 embryonic deaths were higher in the F1 generation than those observed in the F0 generation. For the age group 8 to 14 days, embryonic deaths were higher in the F0 generation than those observed in the F1 generation (Table 9). Analyses of variance for these data are shown in Table 10.

Growth and reproduction data of males were not recorded during the F0 generation. In the F1 generation, inclusion of YSCP in the diets had no significant effect on body weight, feed consumption, percentage of males producing semen or semen volume per ejaculate (Table 11). Analyses of variance for these data are presented in Table 12.

Table 9. Effect of YSCP on distribution of embryonic deaths[a]

	Generation	Replacement of SBM protein, YSCP %				
		00	33	66	100	$\bar{x}$
1-7 days	F0	1.4	1.6	1.4	1.5	1.5±.1
	F1	3.3	3.5	3.7	4.7	3.8**±.3
	$\bar{x}$	2.4±.3[b]	2.5±.3	2.5±.3	3.1±.5	
8-14 days	F0	1.1	1.3	1.1	1.5	1.2±.1
	F1	0.5	0.6	0.3	0.4	0.4**±.1
	$\bar{x}$	0.8±.2	0.9±.2	0.7±.1	0.9±.2	
15-21 days	F0	3.7	2.5	2.2	3.2	2.9±.2
	F1*	9.9	6.7	6.3	5.8	7.2**±.3
	$\bar{x}$*	6.8±.5	4.6±.4	4.3±.4	4.5±.4	

[a]Average for five experimental units of eight hens for the breeder phase of 12, 7-day periods. The data are expressed as percentage of fertile eggs.

[b]Means ± SE.

*Significant effect of levels of YSCP or generations ($P<.05$).

**Significant effect of levels of YSCP or generations ($P<.01$).

Table 10. Analyses of variance of SCWL chickens fed dietary YSCP over two generations

Source of variation	dF		1 to 7	8 to 14	15 to 22
Generation (G)	1		658.8**	76.4**	2173.4**
DIET (D)	3		13.2	1.8	163.5*
Linear	1		31.0	0.2	303.3**
Quadratic	1		5.8	0.3	178.2*
G x D	3		3.0	1.8	67.0
Pen *GxD)	32		31.4	2.7	51.7
Period[a] (P)	11	(1)[b]	15.0	16.0	109.2**
P x G	11	(1)	17.6	8.1	23.6
P x D	33	(3)	10.8	2.6	18.8
P x G x D	33	(3)	8.6	1.5	16.6
Residual	352	(32)	10.2	2.4	16.9
Total	479				

[a]Each period was 7 days in length.

[b]Geisser-Greenhouse procedure for conservative tests was used to find probability of greater F value (Gill, 1978).

*($P \leq .05$).

**($P \leq .01$).

Table 11. Effect of YSCP on performance of SCWL male breeders of F1 generation[a]

	Replacement of SBM protein, YSCP %			
	00	33	66	100
Weight gain, g	433 ±67[b]	500 ±58	566 ±67	533 ±88
Feed consumed, g/male/day	97 ± 3	98 ± 1	97 ± 2	94 ± 3
Males producing semen, %	99 ± 1	98 ± 1	99 ± 1	94 ± 2
Semen/male, Ml	.43± .02	.43± .01	.44± .01	.47± .02

[a]Average for triplicate groups of four males for the breeder phase of 12, 7-day periods.

[b]Means ± SE.

Table 12. Analyses of variance of SCWL roosters fed dietary YSCP (F1 generation)

Source of variation	df		Mean squares: Body wt. gain	Feed intake	Percentage of male producing semen	Semen volume/male
DIET (D)	3		9722.2	11.9	224.2	0.016
Linear	1		20166.6	24.3	313.4	0.040
Quadratic	1		7500.0	11.2	212.7	0.006
Pen (DIET)	8		15000.0	22.0	399.3	0.050
Period[a] (P)	11	(1)[b]			67.5	0.008
D x P	33	(3)			34.8	0.004
Residual	88	(8)			44.2	0.006
Total	143					

[a]Each period was 7 days in length.

[b]Geisser-Greenhouse procedure for conservative tests was used to find probability of greater F value (Gill, 1978).

DISCUSSION

Substituting YSCP for 0, 33, or 66% of the SBM protein supported comparable growth of chicks from hatch to 6 weeks of age. However, a significant reduction in mean ADG was observed during this age period for chicks fed a 100% YSCP diet as compared with that of chicks fed other diets. At the same time, there was a significant reduction in feed intake for this group. Therefore, the decreased ADG observed for chicks fed a 100% YSCP diet can be attributed to the significantly reduced feed intake.

However, the significant reduction in the ADG during the starter phase for the chicks fed a 100% YSCP diet did not have any residual effect on growth of chicks during the grower or breeder phases. At the end of the grower phase, body weights of chickens fed a 100% YSCP diet were similar to those of chickens fed the control diet. These results indicate that chickens fed a 100% YSCP diet compensated for slow growth during the starter phase by growing more rapidly than chickens of other treatment groups during the grower phase.

Feed intake results obtained during the three phases differed primarily in that increased levels of dietary YSCP in the F0 generation had no effect on feed intake whereas, in the F1 generation, increased levels of dietary YSCP significantly reduced feed intake. Although no significant interaction between generation and dietary levels of YSCP was observed for feed intake during the starter phase, there was a

significant interaction between generation and dietary YSCP during the grower and breeder phases. The possible cause for feed intake differences between the F0 and F1 generations may have been the change in ME value of YSCP used in diet formulation. Although it is difficult to separate dietary effect from that of generation because of the experimental design, it seems reasonable that change in ME value of YSCP from 2900 kcal/kg in the F0 generation to 2200 kcal/kg in the F1 generation may have had an influence on the feed intake. Researchers have shown that dietary energy (ME) content seems to be the major factor in regulating feed intake for growing and laying chickens (Scott et al., 1976). When these animals are given diets adequate in all nutrients they will consume a sufficient amount of the diets to obtain a constant intake of ME per day (Scott et al., 1976). Therefore, the differences observed in feed intake between generations F0 and F1 were probably associated with the ME content of the diets. The 2200 kcal ME/kg value of YSCP used in the F1 generation for diet formulation may have been an underestimation of YSCP's ME value. Consequently, the actual dietary ME concentration would have exceeded the expected level, and the chickens of generation F1 would have consumed less feed to satisfy their energy needs than those of the F0 generation.

Efficiency of feed utilization for growth during the grower phase and for egg mass production during the breeder phase was not significantly affected by inclusion of YSCP in the diets. However, a significant interaction was noted between the dietary levels of YSCP and generation

during the starter phase in that the chicks fed a 100% YSCP diet in the FO generation had a poorer feed efficiency than those fed the other dietary treatments. On the other hand, inclusion of YSCP in the diets during the F1 generation had no effect on feed efficiency. This interaction suggests that the diet containing 100% YSCP in the FO generation may have been low in methionine and, consequently, was poorly utilized. This is also supported by the fact that supplementation of additional methionine in the YSCP diets during the F1 generation resulted in a better utilization of the diet per unit of growth.

The growth data recorded in this experiment for replacement pullets do not agree with those reported by Shannon et al. (1976) and Yoshida (1979) for replacement pullets fed 10 or 15% dietary YSCP (n-paraffin), respectively. These researchers found that feeding diets containing 10 or 15% YSCP significantly reduced body weight of pullets at the age of 18 weeks. Results of the present study showed that feeding diets containing up to 13% YSCP had no adverse effect on body weight of pullets to 18 weeks of age.

The significant reduction in ADG observed during the starter phase for chicks fed a 100% YSCP diet did not have a significant carry-over effect on rate of egg production, egg weight or egg yield, although age at 50% egg production was delayed by three days for hens fed a 100% YSCP diet. Similarly, efficiency of feed utilization for egg production was not significantly affected by dietary treatments. The results obtained during the breeder phase of the experiment agree with those obtained

previously when hens were fed YSCP from the same source (Ashraf and Sell, 1981a) and when hens were fed YSCP grown on n-paraffin or on n-alkane (Jackson and Kirkpatrick, 1978; Waldroup and Hazen, 1975).

Hatchability of fertile eggs, hatchability of all eggs incubated and distribution of embryonic deaths were not significantly affected by inclusion of YSCP in the diets. Similarly, neither the percentage of males producing semen nor semen volume per male was significantly affected by dietary treatments during the F1 generation. However, a significant reduction in fertility (3%) was observed for chicks fed 66 or 100% YSCP diets as compared with those fed a 0 or 33% YSCP diet. Although it is difficult to determine whether the reduction in fertility was related to hens or to roosters because of the experimental design, the reduction in fertility may have been related to the roosters. Subjective evaluations made during the semen collection indicated that the semen of roosters fed diets containing 66 or 100% YSCP was thinner in consistency. It seems reasonable that thinner semen may have resulted in a lower concentration of sperm and a reduction in fertility. The effects of dietary YSCP on fertility observed in the present experiment are similar to those reported by VanderWal (1976). VanderWal (1976) also observed a 3% reduction in fertility for chickens fed YSCP (n-paraffin) at the dietary levels of 14% as compared with a control group but this YSCP effect was not statistically significant. Yoshida (1979) obtained different results. He reported that chickens fed a 15% YSCP (n-paraffin) diet over five generations had a significantly higher fertility rate than did chickens fed a control diet.

In general, growth and efficiency of feed utilization during the starter and grower phases and rate of egg production, egg weight, egg yield, efficiency of conversion of feed to egg mass, hatchability of fertile eggs, hatchability of all eggs incubated and distribution of embryonic deaths for chickens fed diets containing YSCP over two generations in the current research were equal to or better than those of chickens fed the control diet. Also, no physical abnormalities in embryos or in live chicks were observed that could be attributed to dietary YSCP. Therefore, these results suggest that the nutritive value of the YSCP tested was comparable to that of SBM.

However, a concern about the use of YSCP in chickens' diets remains. This is related to the significant reduction in fertility observed for chickens fed diets containing 66 or 100% YSCP. The cause of reduction in fertility is not certain. Further research is needed to obtain definitive information on this aspect of YSCP use in diets for reproducing chickens.

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GENERAL SUMMARY AND CONCLUSION

Four trials were conducted to evaluate the nutritional characteristics of a methanol-based yeast single-cell protein (YSCP) for chickens. In experiment 1, the relative availability of phosphorus from YSCP was determined. Chicks, starting at eight days of age, were fed either tricalcium-phosphate (TCP) or YSCP as a source of supplemental phosphorus. The experiment was 14 days in length. The basal diet consisted primarily of corn and isolated soy protein and contained .29% total phosphorus and .76% calcium. The TCP was substituted for limestone, whereas YSCP was substituted for isolated soy protein and cellulose. Each phosphorus source was used in the diets to provide .08, .16, .24, and .32% supplemental phosphorus. Weight gain, percentage femur ash and femur ash were used as criteria of response to dietary phosphorus, and regression analysis was used to evaluate each of these parameters as a function of phosphorus intake. Among the parameters measured, weight of femur ash was most sensitive to change in dietary phosphorus intake. The linear regression equation for TCP and YSCP were $Y = 94.83 + .106X$ and $101.52 + .074X$, respectively, where Y = mg of femur ash and X = mg P intake from TCP or YSCP. Availability of phosphorus from YSCP was estimated according to the slope-ratio technique. The relative availability of phosphorus from YSCP was 69.8% as compared with 100% for TCP.

Experiments 2 and 3 were conducted to evaluate YSCP as a source of amino acids for laying hens. In both experiments, YSCP was substituted

for 0, 33, 66 or 100% of the protein of soybean meal (SBM). The experiments were conducted over eleven 28-day periods. Pooled data for seven periods in experiment 1 showed that inclusion of YSCP in the diets resulted in a decrease in average egg weight, egg yield, feed consumption and body weight gain. Rate of egg production and feed efficiency were not affected by the dietary treatments. At the end of the seventh period, methionine supplementation was increased to .13, .22 and .28% in diets containing 6.55, 12.90 and 19.15% YSCP, respectively. This resulted in an improvement in egg yield for the hens fed 6.55 or 12.9% YSCP diets, but the hens fed 19.15% YSCP diet showed no improvement in egg yield. Body weight gain was improved by increasing the methionine levels of YSCP diets. In experiment 2, mortality increased with increments of dietary YSCP. This relationship was postulated to be the result of a deficiency of selenium (se). Experiment 3 was designed to determine the effect of Se supplementation of YSCP-containing diets on laying hen performance. The dietary treatments consisted of a 2 x 4 factorial arrangement of two levels of Se (0 and .1 ppm) and four levels of dietary YSCP (0, 6.5, 12.7 and 18.7%). These dietary levels of YSCP replaced 0, 33, 66 and 100% of the protein of SBM. Total sulfur amino acid levels of the diets containing 0, 6.5, 12.7 and 18.7% YSCP were .62, .67, .71 and .75, respectively. Neither level of YSCP nor supplemental Se had a significant effect on laying hen performance except for feed intake. Hens fed diets supplemented with Se consumed significantly more feed than those fed nonsupplemented diets. No

relationship was observed between the levels of YSCP or supplemented Se and rate of mortality.

Experiment 4 was conducted to evaluate YSCP as a source of amino acids for SCWL chickens for reproduction over two generations. The experiment was a 2 x 4 factorial arrangement with two generations (FO and F1) and four dietary levels of YSCP. The four dietary levels of YSCP were obtained by substituting YSCP for 0, 33, 66 or 100% of the dietary SBM protein. Each generation was divided into three phases: starter, grower and breeder. Replacing 100% of the SBM protein with YSCP during the starter phase significantly reduced average daily gain (ADG) and feed intake. However, growth during the grower and the breeder phases was not affected by inclusion of YSCP in the diets. Nor was there any effect of dietary YSCP on efficiency of feed utilization during the starter or grower phases. Inclusion of YSCP in the diets during the breeder phase did not significantly affect rate of egg production, egg weight, egg yield, efficiency of conversion of feed into eggs, hatchability of fertile eggs, hatchability of all eggs incubated or distribution of embryonic death. However, substituting YSCP for 66 or 100% of the dietary SBM protein significantly reduced fertility. Growth and reproduction data of males were not recorded during breeder phase of the FO generation. In the F1 generation, inclusion of YSCP in the diets had no significant effect on body weight, feed consumption, percentage of males producing semen or semen volume per ejaculate. No physical abnormalities in embryos or in live chicks were observed that could be attributed to

dietary YSCP. Mortality was low throughout the experiment and was not related to the dietary treatments.

The data presented suggest that the nutritional characteristics of YSCP grown on methanol are equal to or better than those of SBM. YSCP was shown to be a good source of phosphorus for chickens. Also, YSCP was a good source of essential amino acids for laying hens and reproducing chickens, provided that the diets containing YSCP were supplemented with adequate levels of methionine.

Three main concerns about the use of YSCP in laying hens or reproducing chickens' diets remain. These concerns are related to the occasional adverse effects of dietary YSCP on feed intake, mortality and fertility of eggs. More research is needed to obtain definitive information on these aspects of YSCP use in diets for laying hens and reproducing chickens.

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